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(54) Title: A NUCLEIC ACID ENCODING A HUMAN JUNCTIONAL ADHESION PROTEIN (JAM3)

(57) Abstract: The present invention relates to an isolated and purified nucleic acid comprising a polynucleotide encoding for a human junctional adhesion molecule protein and methods for identifying compounds that bind to this protein as well as modulate binding between this protein and other junctional adhesion molecules.

A NUCLEIC ACID ENCODING A HUMAN JUNCTIONAL ADHESION PROTEIN (JAM3)

Technical Field Of The Invention

The present invention relates to molecular biology. More specifically, the present invention related to a nucleic acid comprising a polynucleotide which encodes a human junctional adhesion protein, to a polypeptide encoded by said nucleic acid and to recombinant vectors expressing said polypeptide. Even more specifically, the present invention relates to a direct interaction between two junctional adhesion molecules, referred to as JAM3 and JAM2.

Background Of The Invention

Tight junctions, gap junctions, and adherens junctions contribute to the barrier and communication properties of the vasculature (Lampugnani MG, Dejana, E., *Curr. Opin,. Cell Biol.*, 9:674-682 (1997)). During development, different vascular beds acquire specialized features in accord with organ function (Garlanda, D, Dejana E., *Arterioscler. Thromb. Vasc. Biol.*, 17:1193-1202 (1997)). Heterogeneity extends to the type and extent of inter-cellular contacts that form between opposing endothelial cells (Simionescu M, Simionescu N, Palade GE, *J. Cell Biol.*, 67:863-885 (1975)). This results in varying permeabilities throughout the vascular tree. Tight junctions are crucial structures for maintenance of the blood-brain (hereinafter, "BBB") and blood-retinal (Hereinafter, "BRB") barriers. In recent years, two types of transmembrane protein, namely occludins and claudins, have been described that constitute the tight junction (Fanning AS, Mitic LL, Anderson JM, *J. Am Soc. Nephrol.*, 10:1337-45 (1999)). These proteins possess four putative transmembrane domains. Additionally, occludin can function as an adhesion molecule.

In some instances it may be desirable to selectively disrupt endothelial tight junctions. For example, disruption of the BBB may provide a method for transvascular delivery of therapeutic agents to the brain. (Muldoon LL, Pagel MA, Kroll RA, Roman-Goldstein S, Jones RS, Neuwelt EA, *Am. J. Neuroradiol.*, 20:217-22 (1999)). In another instance, strategies designed to open the tight junctions of polarized epithelial cells may

improve gene delivery for diseases such as cystic fibrosis. In these instances, the polarized apical membranes of airway epithelial cells are resistant to transfection by lipid:pDNA complexes (Chu Q, Tousignant JD, Fand S, Jiang C, Chen LH, Cheng SH, Scheule RK, Eastman SJ, *Hum. Gene. Ther.*, 10:25-26 (1999)).

In addition to regulation of solute permeability, intercellular contacts serve to impede leukocyte egress. However, during inflammation, leukocytes must pass between endothelial cells to reach the interstitial space. Selective recruitment of leukocytes is achieved by differential expression/upregulation of adhesion molecules within the microvasculature (Ley K., *Cardiovasc.R Res.*, 32:733-742 (1996)). Similarly, regional expression of homing receptors facilitates lymphocyte targeting to lymphoid organs (Wiedl G, Dunon D, Imhof BA, *Crit. Rev. Clin. Lab. Sci.*, 38:1-31 (2001)). Adhesion proteins targeted to cell-cell borders are ideally situated to participate in leukocyte emigration. The role of PECAM in this regard is well established (Muller WA, Randolph GJ, *J. Leukoc. Biol.*, 66:698-704 (1999)). More recently, Junctional Adhesion Protein (hereinafter "JAM") has emerged as a player in this arena. Conceivably, differential expression of intercellular adhesion proteins could act as a filter to regulate leukocyte transmigration.

The platelet endothelial cell adhesion molecule, PECAM-1, a member of the Ig superfamily of adhesion proteins, localizes to the lateral membranes between endothelial cells (Zocchi MR, Ferrero E, Leone BE, Rovere P, Bianchi E, Toninelli E, Pardi R, *Euro. J. Immunol.*, 26:759-67 (1996)). However, it is not associated with the tight junction and adherens junction structures (Ayalon O, Sabanai H, Lampugnani MG, Dejana E, Geiger B, *J. Cell. Biol.*, 126(1):247-58 (1994)). The crucial role of PECAM-1 in paracellular migration of leukocytes to extravascular sites has been established (Muller WA, Weigl SA, Deng X, Phillips DM *J. Exp. Med.*, 178:449-60 (1993)).

Partial amino acid sequencing of human Junctional Adhesion Molecule 1 (hereinafter "JAM1") was reported as early as 1995. (Naik UP, Ehrlich YH, Kornecki E, *Biochem. J.*, 310:155-163 (1995)). However, it was not until 1998 that the complete

sequence of a mouse homologue was characterized (Martin-Padura I, Lostaglio S, Scheemann M, Williams L, Romano M, Fruscella P, Panzeri C, Stoppacciaro A, Ruso L, Villa A, Simmons D, Dejana E, *J Cell. Biol.*, 142(1):117-127 (1988)); Malergue F, Galland F, Martin F, Mansuelle P, Aurrand-Lions M, Maquet P, *Mol. Immunol.*, 35:1111-1119 (1998)). The term "junctional adhesion molecule" (JAM) stemmed from its localization at tight junctions in endothelial and epithelial cells. JAM possesses two Ig domains, a single transmembrane and a short intracellular domain. Participation of JAM1 in the inflammatory process was revealed by the ability of a neutralizing monoclonal antibody to modulate monocyte transmigration through the vessel wall. Nevertheless, the ability to inhibit JAM function may allow alleviation of inflammatory diseases such as arthritis, asthma, rheumatoid arthritis, IBD and Crohns. Most recently, JAM1 has been described as a receptor for reovirus (Barton ES, Forrest JC, Connolly JL, Chappell JD, Liu Y, Schnell FJ, Nusrat A, Parkos CA, *Cell*, 104:441-451 (2001)) and LFA-1 (Ostermann G, Weber KS, Zernecke A, Schroder A, Weber C. (2002) *Nat. Immunol.* 3:151-8).

In 2000, Junctional Adhesion Molecule 2 (hereinafter "JAM2") was described by the Applicants of the present invention (Cunningham., S.A., et al., *J. Biol. Chem.*, 275:34750-34756 (2000)). Although JAM2 is expressed at low levels in many tissues, cellular expression appears restricted within the endothelium (Palmeri D, vanZante A, Huang CC, Hemmerich S, Rosen SD, *J. Biol. Chem.*, 275:19139-19145 (2000)). Northern analysis demonstrates that JAM2 is preferentially expressed in the heart. Similar to JAM1, immunolocalization identifies JAM2 at cell-cell borders. However, it remains to be determined whether JAM2 associates with the tight junctional complex. Evidence that JAM2 functions as an adhesion protein has been provided by capture of human T cell lines by JAM2 ectodomain. Applicants have previously shown that this property is mediated, at least in part, by an uncharacterized 43kDa cell surface protein (Cunningham., SA., et al., *J. Biol. Chem.*, 275:34750-34756 (2000)).

Summary Of The Invention

In one embodiment, the present invention relates to an isolated and purified nucleic acid comprising a polynucleotide which encodes a human JAM3 polypeptide.

In yet another embodiment, the present invention relates to an isolated and purified nucleic acid comprising a polynucleotide of SEQ ID NO:1 and conservatively modified and polymorphic variants thereof. In addition, the present invention relates to an isolated and purified nucleic acid comprising a human JAM3 polynucleotide having at least 50%, 70%, 80%, 90% or 95% identity to a polynucleotide of SEQ ID NO:1.

In yet another embodiment, the present invention relates to an isolated and purified polypeptide comprising an amino acid sequence of SEQ ID NO:2. In addition, the present invention relates to an isolated and purified polypeptide comprising an amino acid sequence having at least 60%, 70%, 80%, 90% or 95% identity to an amino acid sequence of SEQ ID NO:2.

In yet a further embodiment, the present invention relates to a recombinant vector comprising an isolated and purified nucleic acid comprising a human JAM3 polynucleotide, wherein said nucleic acid is operably linked to a promoter and a polyadenylation sequence. The promoter used in said recombinant vector can be LTR, SV40, E. Coli, lac , trp, or a phage lambda P_L promoter.

In yet a further embodiment, the present invention relates to a host cell, such as, but not limited to a bacterial cell, a mammalian cell, or a plant cell comprising the recombinant vector.

In yet still a further embodiment, the present invention relates to a transgenic mammal comprising the hereinbefore described recombinant vector.

In yet still a further embodiment, the present invention relates to an antibody which binds to the human JAM3 polypeptide of the present invention.

In yet still a further embodiment, the present invention relates to a method for identifying a compound that binds to junctional adhesion molecule 3. The method involves the steps of contacting JAM3 in the presence of a test compound and detecting the binding between the JAM3 and the test compound.

In yet still a further embodiment, the present invention relates to a method for identifying a compound that modulates binding between JAM2 and JAM3. The method involves the steps of contacting JAM2 and JAM3 in the presence and absence of a test compound; detecting binding between the JAM2 and JAM3 and identifying whether the compound modulates the binding between the JAM2 and JAM3 in view of decreased or increased binding between the JAM2 and JAM3 in the presence of the compound as compared to binding in the absence of the compound.

In yet still a further embodiment, the present invention relates to a method of identifying a compound that modulates binding between an α4β1or α4β7 integrin and JAM2. The method involves contacting α4β1or α4β7 integrin and JAM2 in the presence and absence of a test compound, detecting binding between the α4β1or α4β7 integrin and JAM2, and identifying whether the compound modulates the binding between the α4β1or α4β7 integrin and JAM2 in view of decreased or increased binding between the α4β1or α4β7 integrin and JAM2 in the presence of the compound as compared to binding in the absence of the compound. More specifically, this method can be conducted by immobilizing an α4β1or α4β7 integrin or JAM2 or a fusion protein or fragment thereof on a solid support ("the immobilized binding partner"), labeling the α4β1or α4β7 integrin or JAM2 not immobilized on the solid support ("the non-immobilized binding partner") with a detectable agent, contacting the immobilized binding partner with the labeled non-immobilized binding partner in the presence and absence of a compound capable of specifically reacting with α4β1or α4β7 integrin or JAM2 and optionally in the presence of JAM3, detecting binding between the immobilized binding partner and the non-immobilized binding partner and identifying compounds that affect binding (increase or decrease) between the immobilized binding partner and the labeled non-immobilized binding partner.

In yet still a further embodiment, the present invention relates to a method of inhibiting JAM2-α4β1or α4β7 integrin mediated interactions in a mammal. The method involves the step of administering to a mammal an effective amount of soluble JAM3 or soluble JAM2 to inhibit said interaction.

In yet still a further embodiment, the present invention relates to a method of inhibiting JAM2-α4β1or α4β7 integrin mediated interactions in *in vitro*. The method involves the step of administering to an effective amount of soluble JAM3 or soluble JAM2 to the reaction mixture to inhibit said interaction.

In yet still a further embodiment, the present invention involves a method of preventing JAM2-JAM3 binding a mammal. The method involves administering to an mammal an effective amount of a compound identified previously to prevent said binding.

In yet still a further embodiment, the present invention involves a method of preventing JAM2 mediated interaction with an alpha4 integrin in a mammal. The method involves administering to a mammal an effective amount of a compound identified previously to prevent said binding.

In yet still a further embodiment, the present invention involves a method of preventing JAM2 mediated interaction with an α4β1or α4β7 integrin in a reaction mixture *in vitro*. The method involves administering to the reaction mixture an effective amount of a compound identified previously to prevent said binding.

In yet a further embodiment, the present invention relates to a method of preventing JAM2 – JAM3 interaction in a mammal. The method involves administering to a mammal an effective amount of an anti-JAM2 or anti-JAM3 neutralizing antibody.

Brief Description Of The Figures

Fig. 1 shows the amino acid sequence of Human Junctional Adhesion Molecule 3 (hereinafter "JAM3") polypeptide and its alignment with human JAM2 polypeptide and human JAM1 polypeptide. The sequence was highlighted using BOXSHADE; (▼) - predicted cleavage of signal sequence; (*), conserved cysteine residues; (●), N-linked glycosylation sites (_____), transmembrane domain; (♦), PKC phosphorylation site; (-----), PDZ binding domain.

Fig. 2 shows a Northern Blot Analysis of human JAM3 polypeptide expression. Transcripts were viewed by hybridization to human JAM3 polypeptide (upper panel) and β-actin (lower panel) [α^{32}P]dATP labeled probes. Fig. 2A shows expression in multiple tissues; namely in lane 1, brain; lane 2, heart; lane 3, skeletal muscle; lane 4, colon; lane 5, thymus; lane 6, spleen; lane 7, kidney; lane 8, liver; lane 9, small intestine; lane 10, placenta; lane 11, lung; lane 12, peripheral blood leukocytes. Exposure, 64h. Fig. 2B shows brain specific expression; namely in lane 1, cerebellum; lane 2, cerebral cortex; lane 3, medulla; lane 4, spinal cord; lane 5, occipital lobe; lane 6, frontal lobe; lane 7, temporal lobe; lane 8, putamen. Exposure, 64h. Fig. 2C shows endothelial cell; namely in lane 1, HAEC; lane 2, HUVEC; lane 3, ECV. Exposure, 82h.

Fig. 3 shows screening for human JAM3 polypeptide adhesion to various leukocyte cell lines. Calcein loaded cells were added to immobilized adhesion proteins captured in 96 well plates. Wells were washed, retained cells lysed and fluorescence quantitated with a cytofluor at excitation 485/emission 530nm. HPB-ALL (*A*), HSB (*B*), HL-60 (*C*), K562 (*D*), RAMOS (*E*). Data from a representative experiment. Average ± SEM (n=6).

Fig. 4 shows direct recombinant JAM interactions *in vitro*. Ninety-six (96) wells were coated with cleaved recombinant human JAM3 polypeptide ectodomain and probed for direct binding to Fc fusion of human JAM3 polypeptide, human JAM1 polypeptide, human JAM2 polypeptide, VCAM and IgG2a. Interactions were recorded in a spectrofluor following detection with an alkaline phosphatase conjugated goat anti-mouse IgG2a. A representative experiment. Average ± SEM (n=4).

Fig. 5 shows that JAM3-Fc adheres to Chinese Hamster Ovary (hereinafter "CHO") cell lines expressing JAM2. Calcein loaded stable CHO cell lines, either control (lanes A, C) or expressing full length JAM2 (lanes B,D) were added to immobilized JAM3-Fc (lanes A, B) or JAM1-Fc (lanes C, D) adhesion proteins captured in 96 well plates. Wells were washed, retained cells lysed and fluorescence quantitated with a cytofluor at excitation 485/emission 530nm. Data from a representative experiment. Average ± SEM (n=4).

Fig. 6.shows precipitation of human JAM3 polypeptide from HSB cells by JAM2-Fc. Plasma membranes from HSB cells were surface biotinylated and specific proteins captured with JAM2-Fc and protein A. Protein was retrieved from the complex by elution at pH 9.0, and the sample was either directly analyzed (lane 1) or subjected to a round of immunoprecipitation using anti-JAM3 polypeptide serum (lane 2) or NMS (lane 3). Bands were viewed with streptavidin-HRP and ECL following electrophoresis and transfer.

Fig. 7 shows that soluble human JAM3 polypeptide ectodomain acts as a competitive inhibitor of human JAM2 polypeptide adhesion to T cells. Fig. 7A shows plasma membranes from HSB cells were surface biotinylated and specific proteins captured with JAM2-Fc and protein A in the absence (lane 1) or presence (lane 2) of excess soluble human JAM3 ectodomain. Arrow corresponds to human JAM3 polypeptide. Fig. 7B shows calcein loaded HSB cells were adhered to immobilized JAM2-Fc (lanes A, B) and VCAM-Fc (lanes C, D) in the absence (lanes A, C) or presence (lanes B, D) of excess soluble human JAM3 polypeptide. Wells were washed, retained cells lysed and fluorescence quantitated with a cytofluor at excitation 485/emission 530nm.

Fig. 8A shows the attenuation of JAM2 binding to an α4 integrin. JAM2 adhesion to HSB cells was assessed in TBS and TBS + Mn. The effects of the selective α4 integrin inhibitor, TBC 772 as compared to the control scrambled peptide TBC 1194. Data

expressed as average of cell number bound ± SEM (n=6). Fig 8B shows a dose-response for inhibition of JAM2 adhesion to α4 integrin. JAM2 adhesion to HSB cells was assessed in TBS ( ) and TBS + Mn ( ) under identical conditions to those described for Fig. 8A.

Fig. 9 shows the ability of soluble JAM3 or neutralizing JAM3 antibodies to prevent not only JAM2 adhesion to JAM3, but also JAM2 adhesion to the α4 integrin. JAM2 adhesion to HSB cells was assessed in TBS and TBS + Mn. Additions were either: soluble JAM3 at a 10-fold molar ratio (9A) or a 1:500 dilution of neutralizing JAM3 antiserum (9B). Data expressed as average of cell number bound ± SEM (n=6).

Fig. 10 shows the specific inhibition of JAM2 to integrin using neutralizing antibodies against the α4 integrin and the β1 integrin. JAM2 adhesion to HSB cells was assessed in TBS and TBS + Mn. Neutralizing antibodies (+); isotype controls(-) were preincubated with cells for 30 min prior to addition of cells to JAM2 coated 96 wells. Data expressed as average of cell number bound ± SEM (n=6).

Definitions

Units, prefixes, and symbols are denoted in the SI accepted form. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation, respectively. The headings provided herein are not limitations of the various aspects or embodiments of the invention which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification as a whole.

As used herein, the terms "amplify" or "amplified" as used interchangeably herein refer to the construction of multiple copies of a nucleic acid sequence or multiple copies complementary to the nucleic acid sequence using at least one of the nucleic acid sequences as a template. Amplification methods include the polymerase chain reaction (hereinafter "PCR"; described in U.S. Patent Nos. 5,683,195 and 4,683,202), the ligase chain reaction (hereinafter "LCR"; described in EP-A-320,308 and EP-A-439,182), the

transcription-based amplification system (hereinafter "TAS"), nucleic acid sequence based amplification (hereinafter "NASBA", Cangene, Mississauga, Ontario; described in *Proc. Natl. Acad. Sci., USA*, 87:1874-1878 (1990); *Nature*, 350 (No. 6313): 91-92 (1991)), Q-Beta Replicase systems, and strand displacement amplification (hereinafter "SDA"). The product of amplification is referred to as an amplicon.

As used herein, the term "antibody" includes reference to an immunoglobulin molecule obtained by *in vitro* or *in vivo* generation of a humoral response, and includes both polyclonal and monoclonal antibodies. The term also includes genetically engineered forms such as chimeric antibodies (e.g., humanized murine antibodies), heteroconjugate antibodies (e.g., bispecific antibodies) and recombinant single chain Fc fragments (hereinafter "scFc"). The term "antibody" also includes antigen binding forms of antibodies (e.g., Fab^1, $F(ab^1)_2$, Fab, Fc, and, inverted IgG (See, Pierce Catalog and Handbook, (1994-1995) Pierce Chemical Co., Rockford, IL)). An antibody immunologically reactive with a particular antigen can be generated *in vivo* or by recombinant methods such as by the selection of libraries of recombinant antibodies in phage or similar vectors (see, e.g., Huse et al., *Science*, 246:1275-1281 (1989); and Ward et al., *Nature*, 341:544-546 (1989); and Vaughan et al., *Nature Biotechnology*, 12:309-314) (1996)).

As used herein, the term "conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or conservatively modified variants of the amino acid sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thereupon, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations" and represent one species of conservatively modified variation. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible

"silent variation" of the nucleic acid. One of ordinary skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for the amino acid, methionine; and UGG, which is ordinarily the only codon for the amino acid tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide of the present invention is implicit in each described polypeptide sequence and incorporated herein by reference.

With respect to amino acid sequences, one of skill will recognize that individual substitutions, deletions, or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well-known in the art.

The following six groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Serine (S), Threonine (T);
2) Aspartic acid (D), Glutamic acid (E);
3) Asparagine (N), Glutamine (Q);
4) Arginine (R), Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and
6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W). See also, Creighton, *Proteins*, W. H. Freeman and Company (1984).

As used herein, the term "full length" when used in connection with a specified polynucleotide or encoded protein refers to having the entire amino acid sequence of, a native (i.e., non-synthetic), endogenous, active form of the specified protein. Methods for determining whether a sequence is full-length are well-known in the art. Examples of such methods which can be used include northern or western blots, primer extension, etc.

Additionally, comparison to known full-length homologous sequences can also be used to identify full-length sequences of the present invention.

As used herein, the term "heterologous" when used to describe nucleic acids or polypeptide refers to nucleic acids or polypeptide that originate from a foreign species, or, if from the same species, are substantially modified from their original form. For example, a promoter operably linked to a heterologous structural gene is from a species different from that from which the structural gene was derived, or, if from the same species, is different from any naturally occurring allelic variants.

As used herein, the term "human JAM1 protein" or "human JAM1 polypeptide" refers to the polypeptide shown in Fig. 1 and described in Ozaki H, Ishii K, Horiuchi H, Arai H, Kawamoto T, Okawa K, Iwamatsu A, Kita T., *J. Immunol.*, 163:553-557 (1999).

As used herein, the term "human JAM2 protein" or "human JAM2 polypeptide" refers to the polypeptide shown in Fig. 1 and described in WO 01/14404.

As used herein, the term "human JAM3 gene" refers to a gene of the present invention, specifically, the full genomic sequence encompassing all coding and noncoding sequences complete with regulatory sequences.

As used herein, the term "human JAM3 nucleic acid" refers to a nucleic acid of the present invention, specifically, a nucleic acid comprising a polynucleotide of the present invention encoding a human JAM3 polypeptide (hereinafter "human JAM3 polynucleotide"). An example of a human JAM3 polynucleotide (cDNA) is shown in SEQ ID NO:1.

As used herein, the terms "human JAM3 polypeptide", "human JAM3 peptide" or "human JAM3 protein" as used interchangeable herein refers to a polypeptide shown in SEQ ID NO:2. The term also includes fragments, variants, homologs, alleles or precursors (e.g., preproproteins or proproteins) thereof.

As used herein, the term "hybridization complex" includes reference to a duplex nucleic acid sequence formed by selective hybridization of two single-stranded nucleic acids with each other.

The term "immunologically reactive conditions" as used herein, includes reference to conditions which allow an antibody, generated to a particular epitope of an antigen, to bind to that epitope to a detectably greater degree than the antibody binds to substantially all other epitopes, generally at least two times above background binding, preferably at least five times above background. Immunologically reactive conditions are dependent upon the format of the antibody binding reaction and typically are those utilized in immunoassay protocols.

As used herein, the term "isolated" includes reference to material which is substantially or essentially free from components which normally accompany or interact with it as found in its naturally occurring environment. The isolated material optionally comprises material not found with the material in its natural environment. However, if the material is in its natural environment, the material has been synthetically (e.g., non-naturally) altered by deliberate human intervention to a composition and/or placed in a locus in a cell (e.g., genome or subcellular organelle) not native to a material found in that environment.

Two polynucleotides or polypeptide are said to be "identical" if the sequence of nucleotide or amino acid residues, respectively, in the two sequences is the same when aligned (either manually for visual inspection or via the use of a computer algorithm or program) for maximum correspondence as described below. The terms "identical" or "percent identity" when used in the context of two or more polynucleotide or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence over a comparison window, as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. With respect to polypeptides or proteins having a

"percent identity" or "percentage of sequence identity", one skilled in the art would recognize that residue positions that are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues possessing similar chemical and/or physical properties such as charge or hydrophobicity and therefore do not change the functional properties of the molecule. Where sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well-known to those of skill in the art. Typically, this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. For example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions can be calculated, according to the algorithm of Meyers and Miller, *Computer Applic. Biol. Sci.*, 4:11-17 (1988) e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, Calif., USA).

As used herein, the term "comparison window" includes reference to a contiguous and specified segment of a polynucleotide sequence, wherein the polynucleotide sequence may be compared to a reference sequence and wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (e.g., gaps) compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Generally, the comparison window is at least 20 contiguous nucleotides in length, and can be 30, 40, 50, 100, or even longer. One of ordinary skill in the art would recognize that to avoid a high similarity to a reference sequence due to inclusion of gaps in the polynucleotide sequence a gap penalty is typically introduced and is subtracted from the number of matches.

A sequence comparison between two (2) sequences can be conducted as follows. One sequence functions as a reference sequence and the other sequence as a test sequence. The test sequence is the sequence to be compared with the reference sequence. If a sequence comparison algorithm is to be used, the test and reference sequence are

entered into a computer, subsequence coordinates designated (if necessary) and sequence algorithm program parameters designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequenced based upon the parameters of the program.

The alignment of polynucleotide and/or polypeptide sequences for the purpose of determining sequence identity and similarity can be by either manual alignment and visual inspection or via the use of some type of computer program or algorithm. In fact, a number of computer programs are available which can be used to align polynucleotide and/or polypeptide sequences that are known in the art. For example, the programs available in the Wisconsin Sequence Analysis Package, Version 9 (available from the Genetics Computer Group, Madison, Wisconsin, 52711), such as GAP, BESTFIT, FASTA and TFASTA. For example, the GAP program is capable of calculating both the identity and similarity between two polynucleotide or two polypeptide sequences. Specifically the GAP program uses the algorithm of Needleman and Wunsch (*J. Mol. Biol.*, 48:443-453 (1970)) with the default penalties for gap creation and gap extension set at 50 and 3, for nucleotide alignments, and with the default penalties for gap creation and gap extension set at 12 and 4 for amino acid alignments. Another example of a useful computer program is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments to show relationship and percentage sequence identity. It also plots a tree or dendogram showing the clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng & Doolittle, *J. Mol. Evol.*, 35:351-360 (1987). Yet another example of a useful computer program that can be used to determine percent sequence identity and sequence similarity is the BLAST algorithm (Altsuchul et al., *J. Mol. Biol.*, 215:403-410 (1990)). The software for performing BLAST analysis is publicly available through the National Center for Biotechnology Information ((http://www.ncbi.nlm.nih.gov/).

With respect to polynucleotide sequences, the term "substantial identity" means that the polynucleotide comprises a sequence having about 60%, 70%, 80%, 90% or 95% sequence identity, compared to a reference sequence using one of the alignment programs described herein conducted according to standard parameters. One skilled in the art will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like. Substantial identity of amino acid sequences for these purposes normally means sequence identity of at least about 60%, 70%, 80%, 90% or 95% identity.

Another indication that nucleotide sequences are substantially identical is if two molecules hybridize to each other under stringent conditions. However, nucleic acids which do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This may occur, e.g., when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. One indication that two nucleic acid sequences are substantially identical is that the polypeptide which the first nucleic acid encodes is immunologically cross-reactive with the polypeptide encoded by the second nucleic acid.

With respect to peptides, the term "substantial identity" as used herein means that the peptide comprises a sequence having at least about 60%, 70%, 80%, 90% or 95% sequence identity, to the reference sequence over a specified comparison window. Preferably, optimal alignment is conducted using the homology alignment algorithm of Needleman and Wunsch, *J. Mol. Biol.*, 48:443 (1990). An indication that two peptide sequences are substantially identical is that one peptide is immunologically reactive with antibodies raised against the second peptide. Thus, a peptide is substantially identical to a second peptide, for example, where the two peptides differ only by a conservative substitution. Peptides which are "substantially similar" share sequences as noted above except that residue positions which are not identical may differ by conservative amino acid changes.

As used herein, the term "detection label" refers to a molecule or moiety having a property or characteristic which is capable of detection. A detection label can be directly detectable as with, for example, radioisotopes, fluorophores, chemiluminophores, enzymes, colloidal particles, fluorescent microparticles and the like; or a label may be indirectly detectable as with, for example, specific binding members (such as immobilized or non-immobilized binding partners). It will be understood that direct labels may require additional components such as, for example, substrates, triggering reagents, light, and the like to enable detection of the label. When indirect labels are used for detection, they are typically used in combination with a conjugate. A "conjugate" is typically a specific binding member which has been attached or coupled to a directly detachable label.

As used herein, the term "nucleic acid" refers to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, encompasses known analogues having the essential nature of natural nucleotide in that they hybridize to single-stranded nucleic acids in a manner similar to naturally occurring nucleotide (e.g., peptide nucleic acids).

As used herein, the term "nucleotide(s)" refers to a macromolecule containing a sugar (either a ribose or deoxyribose), a phosphate group and a nitrogenous base.

As used herein, the term "operably linked" includes reference to a functional linkage between a promoter and a second sequence, wherein the promoter sequence initiates and mediates transcription of the DNA sequence corresponding to the second sequence. Generally, operably linked means that the polynucleotide sequence being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in the same reading frame.

As used herein, the term "polynucleotide" refers to a deoxyribopolynucleotide, ribopolynucleotide, or analogs thereof that have the essential nature of a natural

ribonucleotide in that they hybridize, under stringent hybridization conditions to substantially the same nucleotide sequence as naturally occurring nucleotide and/or allow translation into the same amino acid(s) as the naturally occurring nucleotide(s). A polynucleotide can be full length or a subsequence of a native heterologous structural or regulatory gene. Unless otherwise indicated, the term includes reference to the specified sequence as well as the complementary sequence thereof. Thereupon, DNAs or RNAs with backbones modified for stability or for other reasons are "polynucleotides" as that term is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine or modified bases, such as tritylated bases, to name just two examples, are polynucleotides as the term is used herein. As used herein, the term polynucleotide includes such chemically, enzymatically, or metabolically modified forms of polynucleotides, as well as the chemical forms of DNA and RNA characteristics of viruses and cells, including, but not limited to, simple and complex cells.

As used herein, the terms "polypeptide", "peptide", and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residues is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers. The essential nature of such analogues of naturally occurring amino acids is that, when incorporated into a protein, the protein is specifically reactive to antibodies elicited to the same protein but consisting entirely of naturally occurring amino acids. The terms "polypeptide", "peptide", and "protein" are also inclusive of modifications including, but not limited to, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation. Exemplary modifications are described in various texts, such as *Proteins--Structure and Molecular Properties*, 2nd ed., T. E. Creighton, W. H. Freeman and Company, New York (1993), Wold, F., "Post-transnational Protein Modifications: Perspectives and Prospects", pp. 1-12 in *Posttranslational Covalent Modification of Proteins*, B. C. Johnson, Ed., Academic Press, New York (1983); Seifter et al., *Meth. Enzymol.*, 182: 626-646 (1990) and Rattan et al., *Protein Synthesis: Posttranslational Modifications and Aging*, Ann. N.Y. Acad. Sci. 663:48-62 (1992).

As used herein, the term "polymorphic variant" in connection with polynucleotide sequence refers to a variation in the polynucleotide sequence of a particular gene between individuals of a given species. Polymorphic variants also may encompass "single nucleotide polymorphisms" (SNPs), in which the polynucleotide sequence varies by one base. The presence of SNPs may be indicative of, for example, a certain population, a disease state, or a propensity for a disease state.

As used herein, the term "promoter" refers to a region of DNA upstream from the start of transcription and involved in recognition and binding of RNA polymerase and other proteins to initiate transcription. A promoter can optionally include distal enhancers or repressor elements which can be located several thousand base pairs from the start site of transcription.

As used herein, the term "recombinant" includes reference to a cell, or nucleic acid, or vector, that has been modified by the introduction of a heterologous nucleic acid or the alteration of a native nucleic acid to a form not native to that cell, or that the cell is derived from a cell so modified. For example, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed, or not expressed at all.

As used herein, the terms "residue" or "amino acid" or "amino acid residue" are used interchangeably herein to refer to an amino acid that is incorporated into a protein, polypeptide or peptide. The amino acid may be a naturally occurring amino acid, and unless otherwise limited, may encompass known analogs of natural amino acids that can function in a similar manner as naturally occurring amino acids.

As used herein, the terms "selective hybridization" or "selectively hybridizes" include reference to hybridization, under stringent hybridization conditions, or a nucleic acid sequence to a specified nucleic acid target sequence to a detectably greater degree (e.g., at least 2-fold over background) than its hybridization to non-target nucleic acid

sequences and to the substantial exclusion of non-target nucleic acids. Selectively hybridizing sequences typically have about at least about 80%, 90%, 95% or 100% sequence identity (e.g., complementary) with each other.

As used herein, the terms "stringent hybridization conditions" or "stringent conditions" refers to conditions under which a probe will hybridize to its target subsequence, typically in a complex mixture of nucleic acid, but to no other sequences. Stringent conditions are sequence dependent and are different under different environmental parameters. An extensive guide to hybridization of nucleic acids is found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes Part 1*, Chapter 2 "Overview of Principles of Hybridization and the Strategy of Nucleic Acid Probe Assays", Elsevier, New York. Generally, highly stringent conditions are selected to be about 5° C - 10° C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH and nucleic concentration) at which 50% of the target sequence hybridizes to a perfectly matched probe. Stringent conditions are those in which the salt concentration is less than about 1.0M sodium ion, typically about 0.01 to 1.0M sodium ion concentration (or other salts) at a pH of 7.0 to 8.3 and at a temperature of at least about 30° C for short probes (such as those having a length between about 10 to 50 nucleotide) and at least about 60° C for long probes (such as those having a length greater than 50 nucleotides). In contrast, low stringency conditions are at about 15-30° C below the T_m. Stringent hybridization conditions are sequence-dependent and will be different in different circumstances. Longer sequences hybridize at higher temperatures.

SEQUENCE LISTINGS

The present application also contains a sequence listing that contains eighteen (18) sequences. The sequence listing contains nucleotide sequences and amino acid sequences. For the nucleotide sequences, the base pairs are represented by the following base codes:

Symbol	Meaning
A	A; adenine
C	C; cytosine
G	G; guanine
T	T; thymine
U	U; uracil
M	A or C
R	A or G
W	A or T/U
S	C or G

Symbol	Meaning
Y	C or T/U
K	G or T/U
V	A or C or G; not T/U
H	A or C or T/U; not G
D	A or G or T/U; not C
B	C or G or T/U; not A
N	(A or C or G or T/U)

The amino acids shown in the application are in the L-form and are represented by the following amino acid-three letter abbreviations.

Abbreviation	Amino Acid Name
Ala	L-Alanine
Arg	L-Arginine
Asn	L-Asparagine
Asp	L-Aspartic Acid
Asx	L-Aspartic Acid or Asparagine
Cys	L-Cysteine
Glu	L-Glutamic Acid
Gln	L-Glutamine
Glx	L-Glutamine or Glutamic Acid
Gly	L-Glycine

His	L-Histidine
Ile	L-Isoleucine
Leu	L-Leucine
Lys	L-Lysine
Met	L-Methionine
Phe	L-Phenylalanine
Pro	L-Proline
Ser	L-Serine
Thr	L-Threonine
Trp	L-Tryptophan
Tyr	L-Tyrosine
Val	L-Valine
Xaa	L-Unknown or other

Detailed Description Of The Invention

Introduction

The present invention relates to an isolated and purified nucleic acid comprising a polynucleotide sequence which encodes for a human JAM3 polypeptide. In another embodiment, the present invention relates to polypeptides for human JAM3. In yet another embodiment, the present invention relates to recombinant vectors which, upon expression, produce human JAM3. The present invention also relates to host cells transformed with these recombinant vectors.

The present invention also encompasses the adhesion of the JAM3 polypeptide to the JAM2 polypeptide. JAM3 polypeptide may be expressed in T-cells, dendritic cells, natural killer ("NK") cells or endothelial cells and bind to JAM2 polypeptide expressed in endothelial cells. Binding occurs between the JAM2 and JAM3 polypeptide ectodomains in a cation independent manner. The present invention also relates to adhesion of recombinant JAM2 and JAM3 polypeptide ectodomains *in vitro*. In yet another embodiment, the present invention relates to the use of JAM3 or JAM2 polypeptide ectodomains or neutralizing antibodies to bind to endogenous JAM2 or JAM3 polypeptide *in vivo* to perturb function. In yet another embodiment, the present invention relates to the use of JAM3 or JAM2 polypeptide ectodomains or neutralizing antibodies to perturb interactions *in vivo* or *in vitro* between the JAM2-$\alpha 4\beta 1$or $\alpha 4\beta 7$ integrin complex.

Nucleic Acids

In one embodiment, the present invention relates to isolated nucleic acids of DNA, RNA, and analogs and/or chimeras thereof, comprising a polynucleotide, wherein said polynucleotide is a human JAM3 polynucleotide which encodes a polypeptide of SEQ ID NO:2 (a human JAM3 polypeptide) and conservatively modified and polymorphic variants thereof. It is known in the art that the degeneracy of the genetic code allows for a plurality of polynucleotides to encode for the identical amino acid sequence. These "silent variations", as they are commonly referred to, can be used to selectively hybridize and detect allelic variants of the polynucleotides of the present invention.

An example of a human JAM3 polynucleotide which encodes the human JAM3 polypeptide of SEQ ID NO:2 is shown in SEQ ID NO:1. The polynucleotide of SEQ ID NO:1 is 933 base pairs in length.

In another embodiment, the present invention also provides isolated nucleic acids comprising polynucleotides encoding conservatively modified variants of human JAM3 polypeptide of SEQ ID NO:2. Such conservatively modified variants can be used for a number of useful purposes, such as, but not limited to, the generation or selection of antibodies immunoreactive to the non-variant polypeptide. Also, in yet another embodiment, the present invention also relates to isolated nucleic acids comprising polynucleotides encoding one or more polymorphic variants. Polymorphic variants are used to follow the segregation of chromosome regions and are typically used in marker assisted selection methods, which are well-known to one skilled in the art.

In another embodiment, the present invention relates to the isolation of nucleic acids comprising polynucleotides of the present invention which hybridize, under selective hybridization conditions (i.e., stringent hybridization conditions), to the human JAM3 polynucleotide. The isolation of such nucleic acids can be accomplished by a number of techniques. For example, oligonucleotide probes based upon the human JAM3 polynucleotide described herein can be used to identify, isolate or amplify partial

or full-length clones in a deposited library (such as a cDNA or genomic DNA library). For example, a cDNA or genomic library can be screened using a probe based upon the sequence of the human JAM3 polynucleotide described herein. These probes can be used to hybridize with genomic DNA or cDNA sequences to isolate homologous genes in other species, such as other mammals (i.e., mice, rats, primates, canines, etc., bacteria or plants) or additional sequences in the JAM family.

Alternatively, nucleic acids of interest can be amplified from nucleic acid samples using various amplification techniques known in the art. For example, PCR can be used to amplify the sequences of the human JAM3 gene directly from genomic DNA, from cDNA, from genomic libraries or cDNA libraries. PCR and other *in vitro* amplification methods (such as LCR, etc.) can be used to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of the desired mRNA in samples, for nucleic acid sequencing or for other purposes.

In yet another embodiment, the present invention relates to isolated nucleic acid comprising polynucleotides, wherein the polynucleotides of said nucleic acid have a specified identity at the nucleotide level to the previously described human JAM3 polynucleotide. The percentage of identity is at least about 60%, 70%, 80%, 90% or 95%.

In yet another embodiment, the present invention relates to isolated nucleic acids comprising polynucleotides complementary to the previously described human JAM3 polynucleotide. One skilled in the art will recognize that complementary sequences will base pair throughout their entire length with the previously described human JAM3 polynucleotide (meaning that they have 100% sequence identity over their entire length). Complementary bases associate through hydrogen bonding in double stranded nucleic acids. Base pairs known to be complementary include the following: adenine and thymine, guanine and cytosine and adenine and uracil.

In yet another embodiment, the nucleic acid sequences of the present invention can be used to design antisense nucleic acids which can be used to inhibit JAM3 function in various cell systems, such as, but not limited to T-cell adhesion to recombinant JAM2 protein, T-cell migration through endothelial monolayers.

In yet another embodiment, the present invention relates to isolated nucleic acids comprising polynucleotides which comprise at least 15 contiguous bases from the previously described human JAM3 polynucleotide. More specifically, the length of the polynucleotide can be from about 15 contiguous bases to the length of the human JAM3 polynucleotide from which the polynucleotide is a subsequence of. For example, such polynucleotides can be about 15, 25, 35, 45, 55, 65, 75, 85, 95, 100, 200, 400, 500, 750, etc. contiguous nucleotides in length from the previously-described human JAM3 polypeptide. In addition, such sequences can optionally comprise or lack certain structural characteristics from the human JAM3 polynucleotide from which it is derived.

Polypeptides

In one embodiment, the present invention relates to a human JAM3 polypeptide having the amino acid sequence showing in SEQ ID NO:2. The human JAM3 polypeptide is 310 amino acids in length with an estimated molecular weight of approximately 35kDa and isoelectric point of 7.66.

In another embodiment, the present invention relates to a polypeptide having a specified percentage of sequence identity with the human JAM3 polypeptide of the present invention. The percentage of sequence identity is at least about having about 60%, 70%, 80%, 90% or 95%.

The present invention also provides antibodies which specifically react with the human JAM3 polypeptide of the present invention under immunologically reactive conditions. An antibody immunologically reactive with a particular antigen can be generated *in vivo* or by recombinant methods such as by selection of libraries of recombinant antibodies in phage or similar vectors.

Many methods of making antibodies are known to persons skilled in the art. A number of immunogens can be used to produce antibodies specifically reactive to the isolated human JAM3 polypeptide of the present invention under immunologically reactive conditions. An isolated recombinant, synthetic, or native isolated human JAM3 polypeptide of the present invention is the preferred immunogen (antigen) for the production of monoclonal or polyclonal antibodies.

The human JAM3 polypeptide can then be injected into an animal capable of producing antibodies. Either monoclonal or polyclonal antibodies can be generated for subsequent use in immunoassays to measure the presence and quantity of the human JAM3 polypeptide. Methods of producing monoclonal or polyclonal antibodies are known to persons skilled in the art (See, Coligan, *Current Protocols in Immunology* Wiley/Green, NY (1991): Harlow and Lane, *Antibodies: A Laboratory Manual* Cold Spring Harbor Press, NY (1989); and Goding *Monoclonal Antibodies: Principles and Practice* (2d ed.) Academic Press, New York, NY (1986)).

The human JAM3 polypeptide and antibodies can be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionucleotides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, chemiluminescent moieties, magnetic particles, and the like. Patents teaching the use of such labels include U.S. Patent Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241.

The antibodies of the present invention can be used to screen cells and tissues for the expression of the human JAM3 polypeptide of the present invention. The antibodies of the present invention can also be used for affinity chromatography for the purpose of isolating a human JAM3 polypeptide.

The present invention further provides a human JAM3 polypeptide that specifically binds, under immunologically reactive conditions, to an antibody generated against a defined immunogen, such as an immunogen comprising the human JAM3 polypeptide. Immunogens will generally have a length of at least 10 contiguous amino acids from the human JAM3 polypeptide of the present invention, respectively.

As used herein, the term, "specifically binds" includes reference to the preferential association of a ligand, in whole or part, with a particular target molecule (i.e., "binding partner" or "binding moiety" relative to compositions lacking that target molecule). It is, of course, recognized that a certain degree of non-specific interaction may occur between a ligand and a non-target molecule. Nevertheless, specific binding, may be distinguished as mediated through specific recognition of the target molecule. Typically, specific binding results in a much stronger association between the ligand and the target molecule than between the ligand and non-target molecule. Specific binding by an antibody to a protein under such conditions requires an antibody that is selected for its specificity for a particular protein. The affinity constant of the antibody binding site for its cognate monovalent antigen is at least 10^7, usually at least 10^9, more preferably at least 10^{10}, and most preferable at least 10^{11} liters/mole. A variety of immunoassay formats are appropriate for selecting antibodies specifically reactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically reactive with a protein (See Harlow and Lane, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, New York (1988), for a description of immunoassay formats and conditions that can be used to determine specific reactivity). The antibody may be polyclonal but preferably is monoclonal. Generally, antibodies cross-reactive to human JAM3 polypeptide are removed by immunoabsorbtion.

Immunoassays in the competitive binding format are typically used for cross-reactivity determinations. For example, an immunogenic human JAM3 polypeptide can be immobilized to a solid support (an immobilized binding partner). Polypeptides added to the assay compete with the binding of the antisera to the immobilized antigen

(immobilized binding partner). The ability of the above polypeptides to compete with the binding of the antisera to the immobilized human JAM3 polypeptide is compared to the immunogenic human JAM3 polypeptide. The percent cross-reactivity for the above proteins is calculated, using standard calculations known to persons skilled in the art.

The immunoabsorbed and pooled antisera are then used in a competitive binding immunoassay to compare a second "target" polypeptide to the immunogenic polypeptide. In order to make this comparison, the two polypeptides are each assayed at a wide range of concentrations and the amount of each polypeptide required to inhibit 50% of the binding of the antisera to the immobilized protein is determined using standard techniques. If the amount of the target polypeptide required is less than twice the amount of the immunogenic polypeptide that is required, then the target polypeptide is said to specifically bind to an antibody generated to the immunogenic protein. As a final determination of specificity, the pooled antisera is fully immunoabsorbed with the immunogenic polypeptide until no binding to the polypeptide used in the immunoabsorbtion is detectable. The fully immunoabsorbed antisera is then tested for reactivity with the test polypeptide. If no reactivity is observed, then the test polypeptide is specifically bound by the antisera elicited by the immunogenic protein.

The monoclonal or polyclonal antibodies of the present invention can be screened and tested for inhibition of JAM3 adhesion to JAM2, JAM3 adhesion to cells through a putative integrin, inhibition of JAM3 function with regards to leukocyte emigration and adhesion, inhibition / attenuation of the JAM2 /$\alpha 4\beta 1$ or $\alpha 4\beta 7$ interaction, and activation / inhibition of JAM3 intracellular signaling.

Recombinant Vectors

The present invention also related to recombinant vectors which contain the nucleic acids of the present invention, host cells which are genetically engineered with recombinant vectors of the present invention and the production of the polypeptide of the present invention by recombinant techniques.

The nucleic acids of the present invention can be employed for producing polypeptides using recombinant techniques which are well known in the art. For example, the nucleic acids can be included in any one of a variety of expression vectors for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40, bacterial plasmids, phage DNA, baculoviruses, yeast plasmids, vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. One of the most popular vectors for obtaining genetic elements is from the well known cloning of vector pBR322 (available from the American Type Culture Collection, Manassas, Virginia as ATCC Accession Number 37017). The pBR322 "backbone" sections can be combined with an appropriate promoter and the structural sequence to be expressed. However, any other vector may be used as long as it is replicable and viable in the host. The nucleic acids of the present invention may be inserted into one of the hereinbefore mentioned recombinant vectors, in a forward or reverse orientation. A variety of procedures, which are well known in the art, may be used to achieve this. In general, the nucleic acid is inserted into an appropriate restriction endonuclease site(s).

When inserted into an appropriate expression vector, the nucleic acids of the present invention is operatively linked to an appropriate expression control sequence(s), such as a promoter, to direct mRNA synthesis. As used herein, the term "operably linked" includes reference to a functional linkage between a promoter and a second sequence, wherein the promoter sequence initiates and mediates transcription of the nucleic acid sequence corresponding to the second sequence. Generally, operable linked means that the nucleotide sequences are being linked and are contiguous and, where necessary to join two protein coding region, contiguous and in the same reading frame. The heterologous structural sequence can encode a fusion protein including either an N-terminal or C-terminal identification peptide imparting desired characteristics, such as stabilization or simplified purification of expressed recombinant product.

Promoter regions can be selected from any desired gene using chloramphenicol transferase (CAT) vectors or other vectors with selectable markers. Such promoters can

be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), α-factor, acid phosphatase, or heat shock proteins. Examples of bacterial promotors which can be used include, but are not limited to; lacI, lacZ, T3, T7, gpt, lambda P_R, P_L and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Examples of other promoters than can be used include the polyhedrin promoter of baculovirus.

The recombinant expression vector preferably also contains a suitable polyadenylation sequence. Identification of suitable polyadenylation sequences which can be used in said recombinant expression vectors are well known to those skilled in the art. Examples of polyadenylation sequences which can be used included, but are not limited to, the SV40 late polyadenylation sequence and the bovine growth hormone polyadenylation sequence.

Typically, recombinant expression vectors contain an origin of replication to ensure maintenance of the vector. Additionally, such vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells. Examples of selectable marker genes which can be used include, but are not limited to, dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, tetracycline or ampicillin resistance for *E. coli.* and the TRP1 gene for *S. cerevisiae.* The expression vector may also contain a ribosome binding site for translation initiation and a transcription termination segment. The vector may also have appropriate sequences for amplifying expression.

Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described in Sambrook, et al., *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Springs Harbor, N.Y., (1989), which is herein incorporated by reference. Large numbers of suitable vectors and promoters are commercially available and can be used in the present invention. Examples of vectors which can be used include, but are not limited to: Bacterial: pQE70, pQE60, pQE-9

(Qiagen), pBS, pD10, phagescript, psiX174, pBluescript SK, pSKS, pNH8A, pkrH16a, pNH18A (Stratagene); ptrc99a, PKK223-3, pKK233-3, pDR540, pRIT5 (Pharamacia): pGEM (Pormega). Eukaryotic: pWLNEO, pSV2CAT, pG44, pXT1, pSG (Stratagene), pSVK3, pBPV, pMSG, pSVL (Pharmacia).

In another embodiment, the present invention relates to recombinant host cells containing the hereinbefore described recombinant vectors. The vector (such as a cloning or expression vector) containing the hereinbefore described nucleic acid, may be employed to transform, transduce or transfect an appropriate host to permit the host to express the protein. Appropriate hosts which can be used in the present invention, include, but are not limited to prokaryotic cells such as *E. coli,* Streptomyces, *Bacillus subtilis, Salmonella typhimurium,* as well as various species within the general *Pseudomonas, Streptomyces, and Staphylococcus.* Lower eukaryotic cells such as yeast and insect cells such as Drosophilia S2 and Spodoptera Sf9. Introduction of the recombinant construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (see, Davis, L., Dibner, M., Battey, L. *Basic Methods in Molecular Biology*, (1986), herein incorporated by reference).

Various higher eukaryotic cells such as mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of the monkey kidney fibroblasts, described by Gluzman, *Cell*, 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will contain an origin of replication, a suitable promoter and enhancer, and any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites can be used to provide the required nontranscribed genetic elements.

The engineered recombinant host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying genes encoding for the human junctional adhesion protein of the present invention. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression and can be determined experimentally, using techniques which are well known in the art.

Transcription of the nucleic acid encoding the polypeptides of the present invention by higher eukaryotes can be increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA which are about from 10 to about 300 base pairs in length, which act on a promoter to increase its transcription. Examples of suitable enhancers which can be used in the present invention include the SV40 enhancer on the late side of the replication origin base pairs 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (such as temperature shift or chemical induction) and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification. Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents. Such methods are well-known to those skilled in the art.

The polypeptides of the present invention can be recovered and purified from recombinant cell cultures, the cell mass or otherwise according to methods of protein chemistry which are known in the art. For example, ammonium sulfate or ethanol precipitation, acid extraction, and various forms of chromatography e.g., anion/cation exchange, phosphocellulose, hydrophobic interaction, affinity chromatography including immunoaffinity, lectin and hydroxylapatite chromatography. Other methods may include

dialysis, ultrafiltration, gel filtration, SDS-PAGE and isoelectric focusing. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. High performance liquid chromatography (hereinafter, "HPLC") on normal or reverse systems or the like, can be employed for final purification steps.

Binding Activity of JAM3 Polypeptide

The present invention further contemplates that endothelial expressed JAM3 is capable of binding to other yet unidentified integrins. The consensus integrin binding motif is described as (L/I)-(D/E)-(S/T/V)-(P/S) and is normally located within the C-D loop of an Ig fold of an adhesion molecule that engages integrin. Several acidic residues are present within the C-D loop of the second Ig fold of JAM3 that have similarities to this sequence, such as, but not limited to, *N*DVP; *T*DS*R* and *S*ET*G*. A JAM3- integrin interaction is expected to expand the roles and functions that JAM3 plays during leukocyte trafficking and inflammation.

In addition to T cells, the JAM3 polypeptide has been described in dendritic cells and natural killer (NK) cells (Liang, T.W. et al., *J. Immunol.* 168, 1618-1626 (2002)). Dendritic cells are involved in the initial activation of naïve T cells and the interaction of JAM2 located in the high endothelial venules with dendritic cell expressed JAM3 may facilitate emigration of these cells into lymphoid organs. NK cells have the ability to damage a variety of tumor or virally infected cells and recognize cells that fail to express MHC class I molecules. Since JAM2 has been detected within some tumor cell lines, the JAM2 / JAM3 interaction may participate in adhesion of NK cells to their target cell and/or may play a role in inhibition or activation of cytotoxicity. Further, adhesion/activation/inhibition of NK cells can occur following engagement of JAM3 with integrin located on the target cell.

Commonly, IgSF molecules bind integrin through key residues found within the loop(s) intervening the C and D β-strands of the interacting Ig fold. Further, a consensus, L/I-D/E-S/T/V-P/S has been identified from homologous sequences within VCAM-1, ICAMs and MAdCAM-1. The acidic residue is most important to the interaction. By

examining the JAM3 sequence, motifs can be identified that may bind integrin. These are located in the loop between β-strands C and D, of the Ig-fold most proximal to the membrane and are conserved between the human and mouse sequences. The predicted sequences in this loop are shown with highlighting of possible integrin binding sites.

Human JAM3 *R*NDVPLPTDS RANPRFRNSSFHLNSETG
Murine JAM3 *R*NDVPLPTDS RANPRFQNSSFHVNSETG

However, it has been shown for JAM2 interactions with α4β1, that a typical integrin binding motif may not be employed. Thus, a novel binding site may be employed for JAM3/integrin interactions.

Use of JAM3 Polypeptide in Screening Assays

In yet another embodiment, the present invention relates to *in vitro* screening assays for identifying compounds (such as, but not limited to, agonists or antagonists) that are capable of binding to JAM3, activating or inactivating JAM3 or that modulate JAM3 binding to an integrin.

The assays of the present invention can be using techniques known in the art, and preferably are amenable to high-throughput screening of chemical libraries. Suitable candidate compounds that can be used in the methods of the present invention include any molecule, such as, but not limited to, proteins, oligopeptides, small organic molecules, polysaccharides, oligonucleotides (sense or antisense), polynucleotide (sense or antisense), etc. The candidate compound can encompass numerous chemical classes, though they are typically organic molecules. The candidate compound can be obtained for a wide variety of sources including libraries of synthetic or natural compounds. For example, many methods are known in the art for the random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts can be used. Additionally, natural or

synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical methods.

For example, assays to identify candidate compounds that modulate JAM3 binding to an integrin can be conducted as follows. First, a reaction mixture containing JAM3 or integrin is prepared. The reaction mixture is contacted with its binding partner (i.e., if the reaction mixture contains JAM3, then the reaction mixture can be contacted with the integrin). If the reaction mixture contains integrin, then the reaction mixture can be contacted with JAM3) in the presence and absence of the candidate compound under conditions for a time to allow the components of the reaction mixture to interact and bind, thus forming a complex which can be removed and/or detected in the reaction mixture. The candidate compound may be initially included in the reaction mixture or may be added subsequent to the addition of the JAM3 or integrin. A control reaction mixture is incubated without the candidate compound or with a placebo. The formation of any complexes between JAM3 and integrin is detected. The formation of a complex in the control reaction, but not in the reaction mixture containing the candidate compound, indicates that the compound interferes with the binding interaction between JAM3 and the integrin. Additionally, complex formation within reaction mixtures containing the candidate compound and JAM3 or integrin can also be compared to complex formation within reaction mixtures containing the candidate compound and a mutant JAM3 or integrin. This comparison may be useful in identifying compounds that disrupt interactions of mutants but not normal JAM3 or integrin. After the reaction is completed, the unreacted components are removed (such as by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. In view of decreased or increased binding between the JAM3 and integrin in the presence or absence of the candidate compound a determination is made whether or not the candidate compound modulates the binding between the JAM3 and the integrin.

The assays of the present invention can be conducted in heterogenous or homogenous formats. Methodologies using fluorescence polarization (FP) and fluorescence resonance energy transfer (FRET) can be employed. Heterogeneous assays

involve anchoring either JAM3, integrin or the candidate compound onto a solid support (the immobilized binding partner) and then detecting complexes anchored on the solid support at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. With either approach, the order of addition of reactants can be varied to obtain different information about the candidate compounds being tested. For example, candidate compounds that interfere with the interaction between JAM3 and an integrin, by competition, can be identified by conducting the reaction in the presence of the candidate compound, namely, by adding the candidate compound to the reaction mixture prior to or simultaneously with JAM3 and integrin. Alternatively, candidate compounds can be added to the reaction mixture after complexes have been formed.

For example, the JAM3 or an integrin can be immobilized (i.e. anchored) onto a solid support by covalent or non-covalent attachments. Examples of a solid support that can be used include, but are not limited to, glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. The solid support may be spherical, as in a bead (such as, but not limited to a polystyrene or magnetic bead) or microparticle, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod or the wall of a well. Alternatively, the solid support may be flat such a sheet, test strip (such as, but not limited to, a nitrocellulose strip), etc. Those skilled in the art will be able to ascertain the appropriate solid support using routine techniques known in the art.

The non-immobilized component (or non-immobilized binding partner) is modified with a detectable label either directly or indirectly. The immobilized binding partner (either the JAM3 or integrin) is then contacted with the labeled non-immobilized binding partner. If the purpose of the assay is to identify candidate compounds that modulate JAM3 – integrin binding, then the immobilized binding partner is contacted with the labeled binding partner in the presence and absence of candidate compound that is believed to be capable of specifically reacting with JAM3 or integrin. After the reaction is complete, unreacted components are removed (such as by washing with buffers) and any complexes formed remain immobilized on the solid support. The

detection of the complex anchored on the solid surface can be accomplished in a number of ways. Where the non-immobilized binding partner is pre-labeled, the detection of the label immobilized on the surface indicates that complexes were formed. Where the non-immobilized binding partner was not pre-labeled, an indirect detectable label can be used to detect complexes anchored on the solid support, for example, by using a labeled antibody specific for the non-immobilized binding partner (the antibody, may be directly labeled or indirectly labeled with a labeled anti-Ig antibody). Depending upon the order of addition of reaction components, candidate compounds that inhibit complex formation or which disrupt preformed complexes can be detected.

Alternatively, in a homogenous assay: (1) a preformed complex of JAM3 and test solution containing the candidate compound(s) can be prepared in which the test solution containing the candidate compound(s) is modified with a detectable label, but the signal generated by the label is quenched due to complex formation; or (2) a preformed complex of JAM3 and integrin are prepared in which either the JAM3 or integrin are modified with a detectable label, but the signal generated by the label is quenched due to complex formation. The addition of a candidate compound that competes with and displaces one of the species from the preformed complex results in the generation of a signal above background; or (3) the test sample containing the candidate compound(s) can be incubated with soluble JAM3 polypeptide in solution, under conditions that will precipitate any JAM3-ligand complexes that are formed, as known by those skilled in the art. As used herein, the term "soluble JAM3" refers to a JAM3 polypeptide that does not contain a complete transmembrane domain (See Figure 1) but contains the extracellular domain (amino acids 1- 241) of human JAM3. As shown in Figure 1, the transmembrane domain of JAM3 is 23 amino acids in length. Soluble JAM3 may contain no transmembrane domain or may contain a transmembrane domain that is less than the 23 amino acids in length. The precipitated complexes are then separated from the test sample, using methods known in the art such as centrifugation.

Using other methodologies, in a homogeneous assay, JAM3 and integrin can be conjugated, either directly or indirectly to donor and acceptor fluorophore beads

respectively, such as those composing the AlphaScreen technology. Upon adhesion of JAM3 with integrin, the beads will be brought into proximity. A laser is employed to excite for example the donor bead conjugated to JAM3, which results in conversion of ambient oxygen to a more excited singlet state. The singlet state oxygen molecules diffuse across and react with a chemiluminescer in the acceptor bead conjugated to integrin. Further activation of fluorophores contained within the same bead, emit light at 520-620 nm.

Using other methodologies, JAM3 or integrin may be fused with each of EGFP (enhanced green fluorescent protein) and EBFP (enhanced blue fluorescent protein). EBFP and EGFP mutants of GFP, when in close proximity to one another and can act as a fluorescence resonance energy transfer (FRET) pair. Thus, upon mixing of JAM3 and integrin complexes will form. Excitation of the JAM-EBFP will result in emissions that excite the JAM-EGFP, and thus the extent of complex formation can be monitored in the presence or absence of compound.

The JAM3 and/or integrin used in the assays of the present invention can be prepared using recombinant DNA techniques described herein. Additionally, JAM3 and/or integrin fusion proteins and fragments of JAM3 and/or integrin that correspond to the binding domains of JAM3 and/or integrin can also be used in the assays described herein. Alternatively, the JAM3 and/or integrin used in the assay described herein can be expressed on the surface of a cell.

Screening Assays for Identifying Compounds that Modulate JAM2-JAM3 Binding

In yet another embodiment, the present invention relates to *in vitro* screening assays for identifying compounds (such as, but not limited to, small molecule inhibitors) that disrupt or modulate JAM2-JAM3 binding. The assays of the present invention can be conducted using techniques known in the art, and preferably are amenable to high-throughput screening of chemical libraries. Suitable candidate compounds that can be used in the methods of the present invention include any molecule, such as, but not limited to, proteins, oligopeptides, small organic molecules, polysaccharides, oligonucleotides (sense or antisense), polynucleotide (sense or antisense), etc. The

candidate compound can encompass numerous chemical classes, though they are typically organic molecules. The candidate compound can be obtained for a wide variety of sources including libraries of synthetic or natural compounds. For example, many methods are known in the art for the random and directed synthesis of a wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts can be used. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical methods.

The assays of the present invention involve preparing a reaction mixture containing either JAM2 or JAM3. The reaction mixture is contacted with its binding partner (i.e., if the reaction mixture contains JAM2, then the reaction mixture can be contacted with JAM3). If the reaction mixture contains JAM3, then the reaction mixture can be contacted with JAM2) in the presence and absence of the candidate compound under conditions for a time to allow the components of the reaction mixture to interact and bind, thus forming a complex which can be removed and/or detected in the reaction mixture. The candidate compound may be initially included in the reaction mixture or may be added subsequent to the addition of the JAM2 or JAM3. A control reaction mixture is incubated without the candidate compound or with a placebo. The formation of any complexes between JAM2 and JAM3 is detected. The formation of a complex in the control reaction, but not in the reaction mixture containing the candidate compound, indicates that the compound interferes with the binding interaction between JAM2 and the JAM3. Additionally, complex formation within reaction mixtures containing the candidate compound and JAM2 or JAM3 can also be compared to complex formation within reaction mixtures containing the candidate compound and a mutant JAM2 or JAM3. This comparison may be useful in identifying compounds that disrupt interactions of mutants but not normal JAM2 or JAM3. After the reaction is completed, the unreacted components are removed (such as by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. In view of decreased or increased binding between the JAM2 and JAM3 in the presence or absence

of the candidate compound a determination is made whether or not the candidate compound modulates the binding between the JAM2 and the JAM3.

The assays of the present invention can be conducted in a heterogeneous or homogeneous format. Heterogeneous assays involve anchoring either the JAM2 or JAM3 onto a solid support and then detecting complexes anchored on the solid support at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. With either approach, the order of addition of reactants can be varied to obtain different information about the candidate compounds being tested. For example, candidate compounds that interfere with the interaction between JAM2 and JAM3, by competition, can be identified by conducting the reaction in the presence of the candidate compound, namely, by adding the candidate compound to the reaction mixture prior to or simultaneously with JAM2 and JAM3. Alternatively, candidate compounds can be added to the reaction mixture after complexes have been formed.

For example, in a heterogeneous assay, JAM2 or JAM3 is immobilized (i.e. anchored) on a solid support by covalent or non-covalent attachments (the immobilized binding partner Examples of a solid support that can be used include, but are not limited to, glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. The solid support may be spherical, as in a bead (such as, but not limited to a polystyrene or magnetic bead) or microparticle, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod or the wall of a well. Alternatively, the solid support may be flat such a sheet, test strip (such as, but not limited to, a nitrocellulose strip), etc. Those skilled in the art will be able to ascertain the appropriate solid support using routine techniques known in the art.

The non-immobilized component (or non-immobilized binding partner) is modified with a detectable label either directly or indirectly. The immobilized binding partner (either the JAM2 or JAM3) is then contacted with the labeled binding partner in the presence and absence of candidate compound that is believed to be capable of

specifically reacting with JAM2 or JAM3. After the reaction is complete, unreacted components are removed (such as by washing with buffers) and any complexes formed remain immobilized on the solid support. The detection of the complex anchored on the solid surface can be accomplished in a number of ways. Where the non-immobilized binding partner is pre-labeled, the detection of the label immobilized on the surface indicates that complexes were formed. Where the non-immobilized binding partner was not pre-labeled, an indirect detectable label can be used to detect complexes anchored on the solid support, for example, by using a labeled antibody specific for the non-immobilized binding partner (the antibody, may be directly labeled or indirectly labeled with a labeled anti-Ig antibody). Depending upon the order of addition of reaction components, candidate compounds that inhibit complex formation or which disrupt preformed complexes can be detected.

Alternatively, the reaction can be conducted in a liquid phase in the presence or absence of the candidate compound, the reaction products separated from unreacted components, and complexes detected. For example, the complexes can be detected using an immobilized antibody specific for one of the binding components to anchor any complexes formed in solution, and a labeled antibody specific for the other partner to detect immobilized complexes. Depending upon the order of addition of reactants to the liquid phase, test compounds which inhibit complex or which disrupt preformed complexes can be identified.

For example, in a homogeneous assay, a preformed complex of JAM2 and JAM3 are prepared in which either the JAM2 or JAM3 modified with a detectable label, but the signal generated by the label is quenched due to complex formation. The addition of a candidate compound that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above background. Therefore, candidate compounds that disrupt JAM2-JAM3 heterotypic binding interaction can be identified.

Using other methodologies, in a homogeneous assay, JAM2 and JAM3 can be conjugated, either directly or indirectly to donor and acceptor fluorophore beads respectively, such as those composing the AlphaScreen technology. Upon adhesion of JAM2 with JAM3, the beads will be brought into proximity. A laser is employed to excite for example the donor bead conjugated to JAM2 and/or JAM3, which results in conversion of ambient oxygen to a more excited singlet state. The singlet state oxygen molecules diffuse across and react with a chemiluminescer in the acceptor bead conjugated to integrin. Further activation of fluorophores contained within the same bead, emit light at 520-620 nm.

Using other methodologies, JAM2 and JAM3 may be fused with each of EGFP (enhanced green fluorescent protein) and EBFP (enhanced blue fluorescent protein). EBFP and EGFP mutants of GFP, when in close proximity to one another and can act as a fluorescence resonance energy transfer (FRET) pair. Thus, upon mixing of JAM2 and JAM3 complexes will form. Excitation of the JAM-EBFP will result in emissions that excite the JAM-EGFP, and thus the extent of complex formation can be monitored in the presence or absence of compound.

The JAM2 and JAM3 used in the assays of the present invention can be prepared using recombinant DNA techniques described herein. Additionally, JAM2 and/or JAM3 fusion proteins and fragments of JAM2 and/or JAM3 that correspond to the binding domains of JAM2 and/or JAM3 can also be used in the assays described herein. Alternatively, the JAM2 and/or JAM3 used in the assay described herein can be expressed on the surface of a cell.

JAM2 Interaction with the α4β1 Integrin

As demonstrated in the Examples, JAM2 exhibits strong heterotypic binding to JAM3. JAM2 also exhibits heterotypic binding to the α4β1 integrin. More specifically, the inventors have discovered that when JAM2 binds to cell surface JAM3, JAM2 also becomes available to bind to α4β1 integrin. Therefore, JAM3 may act as a co-factor for JAM2 binding. Under the assay conditions of the present invention the binding of JAM2

to α4β1 integrin does not appear to occur unless JAM2 has bound to JAM3 on the same cell membrane. As used herein, the term "α4β1 integrin" means an integrin heterodimer where the α4 subunit non-covalently associates with a β1 subunit. It is believed that JAM2 may also exhibit similar binding to the α4β7 integrin.

While not wishing to be bound by any theory, the inventors believe that the binding between JAM2 and α4β1 integrin on the cell membrane may be the result of: (a) prior adhesion of JAM3, expressed on the cell surface, with immobilized JAM2, allowing enhancement of a lower affinity interaction between JAM2 and α4β1 by allowing α4β1, closer more frequent contacts with JAM2; (b) a conformation change that occurs on JAM2 after binding with JAM3 that allows its subsequent interaction with the integrin; (c) that the adhesion of JAM2 with JAM3 initiates clustering of the integrin and presentation to JAM2; (d) that JAM3 directly associates with the integrin through low affinity interactions, and that this may be regulated via JAM2/JAM3 binding; adhesion of JAM2 to JAM3 induces a conformational change of the integrin allowing its interaction with JAM2; or (e) following JAM2 adhesion to JAM3, an intracellular signaling cascade is activated that results in integrin "inside-out" signaling.

Use of Soluble JAM2 or JAM3 to Prevent JAM2-α4β1 or α4β7 Integrin Binding or Interaction

In yet another embodiment, the present invention relates to the *in vivo* or *in vitro* use of an effective amount of soluble JAM2 or JAM3 to prevent JAM2-α4β1 or JAM2-α4β7 integrin binding or interaction. As used herein, the term "soluble JAM2" refers to a JAM2 polypeptide that does not contain a complete transmembrane domain (See Figure 1) but contains the extracellular domain (amino acids 1-236) of human JAM2 or fragments thereof. As shown in Figure 1, the transmembrane domain of JAM2 is 23 amino acids in length. Soluble JAM2 may contain no transmembrane domain or may contain a transmembrane domain that is less than 23 amino acids in length.

Soluble JAM2 or soluble JAM3 can be obtained using techniques known in the art. Specifically, soluble JAM2 or soluble JAM3 can be isolated and purified in its native

form, using techniques known in the art. Alternatively, soluble JAM2 or soluble JAM3 can be chemically synthesized using methods known in the art, preferably solid state methods, such as the methods of Merrifield (*J. Am. Chem. Soc.*, 85:2149-2154 (1963)). Additionally, soluble JAM2 or soluble JAM3 can be produced using methods of DNA recombinant technology (Sambrook et al., in "Molecular Cloning – A Laboratory Manual", 2ND. Ed., Cold Spring Harbor Laboratory (1989)). For example, recombinant cells lines can be used. Specifically, cells expressing soluble JAM3 or soluble JAM2 polypeptide can be labeled with a signal generating compound using techniques known in the art. Suitable signal generating compounds are known in the art. Furthermore, soluble JAM2 or soluble JAM3 can be fused to an Fc region of an immunoglobulin molecule, such as, but not limited to a mouse or human IgG, to make a recombinant fusion protein. Alternatively, soluble JAM2 or soluble JAM3 can be fused to alkaline phosphate or GFP to make a recombinant fusion protein. Finally, cell-free translation can also be employed to produce such JAM2 or JAM3 using RNAs derived from the DNA constructs of the present invention.

An effective amount of soluble JAM2, soluble JAM3 or a recombinant fusion protein thereof can be administered either *in vivo* (such as to a mammal (such as a human patient) in need of treatment) or *in vitro* (such as in an assay), to adhere to cell surface expressed JAM2 or JAM3. Possible interactions may occur, including JAM2 or JAM3 binding to cell surface expressed JAM2, JAM3 or JAM2 binding to cell surface expressed JAM3. Heterotypic adhesion of soluble JAMs with cell surface JAM2 or JAM3 will prevent subsequent heterotypic interaction of JAMs between cells. Similarly, homotypic adhesion of soluble JAMs with cell surface expressed JAM2 or JAM3, may result in modulation of homotypic / heterotypic interaction of JAMs between cells. When the JAM2-JAM3 interaction does not occur, JAM2 binding to $\alpha 4\beta 1$ or $\alpha 4\beta 7$ is not apparent. When this JAM2-JAM3 interaction does not occur, endogenous JAM2 is appears less able unable to bind $\alpha 4\beta 1$ or $\alpha 4\beta 7$. As used herein, the term "cell surface bound JAM3", refers to the expression of JAM3 in any cell type that is adhering to a JAM2 expressing cell. The cells expressing endogenous JAM2 and JAM3 may be of the same type i.e. both may be endothelial cells. Alternatively, the cell types may be

different. For example, one may be an endothelial cell and one may be a leukocyte. Moreover, the adhesion of JAM2 with JAM3 laterally, within the same cell may occur. Soluble JAM2 or soluble JAM3 can also be administered to prevent this interaction as well. Additionally, an effective amount of soluble JAM3 can be administered to a mammal for the purpose of preventing T-cell, NK cell or dendritic cell migration. Soluble JAM3 would bind to endothelial cells expressed by JAM2 thereby preventing adhesion of T-cell expressed JAM3. Alternatively, an effective amount of soluble JAM2 can be administered to a mammal for the purpose of binding to T-cell expressed JAM3 to prevent T-cell adhesion and/or migration to endothelium.

An effective amount of soluble JAM2 or soluble JAM3 that is administered to a mammal for the purposes described above can be determined by those of ordinary skill using routine techniques known in the art. The exact dose will be ascertainable by one skilled in the art. As known in the art, adjustments based on age, body weight, sex, diet, time of administration, drug interaction and severity of condition may be necessary and will be ascertainable with routine experimentation by those skilled in the art.

Screening Assays for Identifying Compounds that Modulate JAM2-α4β1/α4β7 Integrin Binding

In yet another embodiment, the present invention relates to *in vitro* screening assays for identifying compounds (such as, but not limited to, small molecule inhibitors) that disrupt or modulate JAM2-α4β1/α4β7 integrin binding. The assays of the present invention can be conducted using techniques known in the art, and preferably are amenable to high-throughput screening of chemical libraries. Suitable candidate compounds that can be used in the methods of the present invention include any molecule, such as, but not limited to, proteins, oligopeptides, small organic molecules, polysaccharides, oligonucleotides (sense or antisense), polynucleotide (sense or antisense), etc. The candidate compound can encompass numerous chemical classes, though they are typically organic molecules. The candidate compound can be obtained from a wide variety of sources including libraries of synthetic or natural compounds. For example, many methods are known in the art for the random and directed synthesis of a

wide variety of organic compounds and biomolecules, including expression of randomized oligonucleotides. Alternatively, libraries of natural compounds in the form of bacterial, fungal, plant and animal extracts can be used. Additionally, natural or synthetically produced libraries and compounds are readily modified through conventional chemical, physical and biochemical methods.

The assays of the present invention involve preparing a reaction mixture containing either an α4β1/α4β7 integrin or JAM2. Optionally, the reaction mixture can also contain JAM3. The reaction mixture is contacted with its binding partner (i.e., if the reaction mixture contains JAM2, then the reaction mixture can be contacted with an α4β1/α4β7 integrin. If the reaction mixture contains an α4β1/α4β7 integrin, then the reaction mixture can be contacted with JAM2) in the presence and absence of the candidate compound under conditions for a time to allow the components of the reaction mixture to interact and bind, thus forming a complex which can be removed and/or detected in the reaction mixture. The candidate compound may be initially included in the reaction mixture or may be added subsequent to the addition of the JAM2 and α4β1/α4β7 integrin. A control reaction mixture is incubated without the candidate compound or with a placebo. The formation of any complexes between JAM2 and the α4β1/α4β7 integrin is detected. The formation of a complex in the control reaction, but not in the reaction mixture containing the candidate compound, indicates that the compound interferes with the binding interaction between JAM2 and the α4β1/α4β7 integrin. Additionally, complex formation within reaction mixtures containing the candidate compound and JAM2 or α4β1/α4β7 integrin can also be compared to complex formation within reaction mixtures containing the candidate compound and a mutant JAM2 or α4β1/α4β7 integrin. This comparison may be useful in identifying compounds that disrupt interactions of mutants but not wildtype JAM2 or α4β1/α4β7 integrin. After the reaction is completed, the unreacted components are removed (such as by washing) under conditions such that any complexes formed will remain immobilized on the solid surface. In view of decreased or increased binding between the α4β1/α4β7 integrin and JAM2 in the presence or absence of the candidate compound a determination is made whether or not

the candidate compound modulates the binding between the JAM2 and the α4β1/α4β7 integrin.

The assays of the present invention can be conducted in a heterogeneous or homogeneous format. Heterogeneous assays involve anchoring either the JAM2 or α4β1/α4β7 integrin onto a solid support and then detecting complexes anchored on the solid support at the end of the reaction. In homogeneous assays, the entire reaction is carried out in a liquid phase. With either approach, the order of addition of reactants can be varied to obtain different information about the candidate compounds being tested. For example, candidate compounds that interfere with the interaction between JAM2 and α4β1/α4β7 integrins, by competition, can be identified by conducting the reaction in the presence of the candidate compound, namely, by adding the candidate compound to the reaction mixture prior to or simultaneously with JAM2 and α4β1/α4β7 integrin. Alternatively, candidate compounds can be added to the reaction mixture after complexes have been formed. Moreover, with either approach, JAM3 can be added to the reaction to improve the specificity and/or sensitivity of the reaction.

For example, in a heterogeneous assay, JAM2 or α4β1/α4β7 integrin is immobilized (i.e. anchored) on a solid support by covalent or non-covalent attachments (the immobilized binding partner). Examples of a solid support that can be used include, but are not limited to, glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. The solid support may be spherical, as in a bead (such as, but not limited to a polystyrene or magnetic bead) or microparticle, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod or the wall of a well. Alternatively, the solid support may be flat such a sheet, test strip (such as, but not limited to, a nitrocellulose strip), etc. Those skilled in the art will be able to ascertain the appropriate solid support using routine techniques known in the art.

The non-immobilized component (or non-immobilized binding partner) is modified with a detectable label either directly or indirectly. The immobilized binding

partner (either the JAM2 or α4β1/α4β7 integrin) is then contacted with the labeled binding partner in the presence and absence of candidate compound that is believed to be capable of specifically reacting with JAM2 or α4β1/α4β7 integrin. After the reaction is complete, unreacted components are removed (such as by washing with buffers) and any complexes formed remain immobilized on the solid support. The detection of the complex anchored on the solid surface can be accomplished in a number of ways. Where the non-immobilized binding partner is pre-labeled, the detection of the label immobilized on the surface indicates that complexes were formed. Where the non-immobilized binding partner was not pre-labeled, an indirect label can be used to detect complexes anchored on the solid support, for example, by using a labeled antibody specific for the non-immobilized binding partner (the antibody, may be directly labeled or indirectly labeled with a labeled anti-Ig antibody). Depending upon the order of addition of reaction components, candidate compounds that inhibit complex formation or which disrupt preformed complexes can be detected.

Alternatively, the reaction can be conducted in a liquid phase in the presence or absence of the candidate compound, the reaction products separated from unreacted components, and complexes detected. For example, the complexes can be detected using an immobilized antibody specific for one of the binding components to anchor any complexes formed in solution, and a labeled antibody specific for the other partner to detect immobilized complexes. Depending upon the order of addition of reactants to the liquid phase, test compounds which inhibit complex or which disrupt preformed complexes can be identified.

For example, in a homogeneous assay, a preformed complex of JAM2 and α4β1/α4β7 integrin are prepared in which either the JAM2 or α4β1/α4β7 integrin are modified with a label, but the signal generated by the label is quenched due to complex formation. The addition of a candidate compound that competes with and displaces one of the species from the preformed complex will result in the generation of a signal above background. Therefore, candidate compounds that disrupt the JAM2/ α4β1 or α4β7 integrin binding interaction can be identified.

Using other methodologies, in a homogeneous assay, JAM2 and α4β1 or α4β7 integrin can be conjugated, either directly or indirectly to donor and acceptor fluorophore beads respectively, such as those composing the AlphaScreen technology. Upon adhesion of JAM2 with integrin, the beads will be brought into proximity. A laser is employed to excite for example the donor bead conjugated to JAM2, which results in conversion of ambient oxygen to a more excited singlet state. The singlet state oxygen molecules diffuse across and react with a chemiluminescer in the acceptor bead conjugated to integrin. Further activation of fluorophores contained within the same bead, emit light at 520-620 nm.

Using other methodologies, JAM2 and α4β1/α4β7 integrin may be fused with each of EGFP (enhanced green fluorescent protein) and EBFP (enhanced blue fluorescent protein). EBFP and EGFP mutants of GFP, when in close proximity to one another and can act as a fluorescence resonance energy transfer (FRET) pair. Thus, upon mixing of JAM2 and α4β1/α4β7 integrin complexes will form. Excitation of the JAM-EBFP will result in emissions that excite the JAM-EGFP, and thus the extent of complex formation can be monitored in the presence or absence of compound.

The JAM2 and/or α4β1/α4β7 integrin and optionally, JAM3 used in the assays of the present invention can be prepared using recombinant DNA techniques described herein. For example, recombinant cells expressing JAM2, and/or α4β1/α4β7 integrin and optionally JAM3 can be used. Alternatively, JAM2 or JAM3 can be isolated and purified in its native form or chemically synthesized using methods known in the art. Additionally, JAM2 and/or α4β1/α4β7 integrin fusion proteins and fragments of JAM2 and/or α4β1/α4β7 integrin that correspond to the binding domains of JAM2 and/or α4β1/α4β7 integrin can also be used in the assays described herein. Alternatively, the JAM2 and/or α4β1/α4β7 integrin and optionally, JAM3 used in the assay described herein can be expressed on the surface of a cell.

Pharmaceutical Compositions

In another embodiment, the present invention relates to certain compounds and pharmaceutical compositions that can be administered to a mammal in need of treatment. One type of compound that can be administered to a mammal alone or in a pharmaceutical composition, where it is mixed with suitable carriers or excipients, is soluble JAM2 or soluble JAM3. Soluble JAM2 or soluble JAM3 modulates or interferes with JAM2- $\alpha 4\beta 1/\alpha 4\beta 7$ integrin binding. Soluble JAM3 modulates or interferes with JAM3 integrin binding.

Soluble JAM2 or soluble JAM3 can be isolated and purified in its native form, using techniques known in the art. Alternatively, soluble JAM2 or soluble JAM3 can be chemically synthesized using methods known in the art, preferably solid state methods, such as the methods of Merrifield (*J. Am. Chem. Soc.*, 85:2149-2154 (1963)). Additionally, soluble JAM2 or soluble JAM3 can be produced using methods of DNA recombinant technology (Sambrook et al., in "Molecular Cloning – A Laboratory Manual", 2^{ND}. Ed., Cold Spring Harbor Laboratory (1989)). Additionally, soluble JAM2 or soluble JAM3 fusion proteins or fragments of soluble JAM2 or soluble JAM3 can also be used. For example, peptides 5-25 amino acids in length, linear or cyclized, derived from the sequences of JAM2 and JAM3, that are found to prevent either the adhesion of JAM2 with JAM3, or the adhesion of JAM2 with $\alpha 4\beta 1$, can be used. These fusion proteins or fragments of JAM2 or JAM3 can be made using the techniques described herein. Other compounds that can be administered to a mammal alone or in a pharmaceutical composition are the candidate compounds identified pursuant to the assays described herein.

Suitable excipients that can be used in the pharmaceutical composition of the present invention, include but are not limited to, fillers such as sugars, including lactose, sucrose, mannitol, sorbitol, and the like, cellulose preparations such as, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, ethyl cellulose, hydroxypropylmethylcellulose, sodium carboxymethylcellulose, polyvinylpyrrolidone (PVP), and the like, as well as mixtures of any two or more. Optionally, disintegrating

agents can be included, such as cross-linked polyvinyl pyrrolidone, agar, alginic acid or a salt thereof, such as sodium alginate and the like.

In addition to the excipients, the pharmaceutical composition can include one or more of the following, carrier proteins such as serum albumin, buffers, binding agents, sweeteners and other flavoring agents; coloring agents and polyethylene glycol.

Suitable routes of administration for the compound or pharmaceutical composition include, but are not limited to, oral, rectal, transdermal, vaginal, transmucosal or intestinal administration, parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, intraocular, and the like.

For oral administration, the pharmaceutical composition can be formulated as tablets, pills, capsules, dragees, liquids, gels, syrups, slurries, suspensions and the like. For administration by injection, the compound or the pharmaceutical composition can be formulated in an aqueous solution. Preferably, the aqueous solution is in a physiologically compatible buffer such as Hank's solution, ringer's solution or a physiological saline buffer.

The pharmaceutical composition of the present invention can be manufactured using techniques known in the art, such as, but not limited to, conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping, lyosphilizing processes or the like.

Methods of Treatment

The present invention also relates to methods of treating a mammal with the previously described compounds or pharmaceutical compositions. The administration of these compounds may be for the purpose of binding to and/or activating or inactivating the JAM3 polypeptide, preventing JAM3 – integrin mediated interaction or preventing JAM2-JAM3 interaction or for preventing the JAM2 - $\alpha 4\beta 1/\alpha 4\beta 7$ integrin mediated interaction. Alternatively, effective amounts of anti-JAM2 or anti-JAM3 neutralizing

antibodies can also be administered to a mammal for the purpose of preventing JAM2-JAM3 interaction. It is believed that preventing these interactions can be used to treat diseases associated with leukocyte adhesion to the endothelium and their subsequent diapedesis through the vessel wall. This phenomenon commonly occurs during inflammation and accompanies autoimmune diseases. Therefore, the compounds identified pursuant to the assays of the present invention can be used to treat conditions or disorders, including, but not limited to, systemic lupus erythematosis, rheumatoid arthritis, juvenile chronic arthritis, spondyloarthropathies, systemic sclerosis (scleroderma), idiopathic inflammatory myopathies (including, dermatomyositis, polymyositis, etc), Sjsgren's syndrome, systemic vasculitis, sarcoidosis, autoimmune hemolytic anemia (including, immune pancytopenia, paroxysmal nocturnal hemoglobinuria), autoimmune thrombocytopenia (including idiopathic thrombocytopenic purpurn, immune-mediated thrombocytopenia), thryoiditis (including Grave's disease, Hashimoto's thyroiditis, juvenile lymphocytic thyroiditis, atrophic thyroiditis), diabetes mellitus, immune-mediated renal disease (including glomerulonephritis, tubulinterstitial nephritis), demyelinating diseases of the central and peripheral nervous system, including multiple sclerosis, idiopathic demyelinating polyneuropathy or Guillain-Barre syndrome, chronic inflammatory demyelinating polyneuropathy), hepatobiliary diseases (including hepatitis A-E and other non-hepatotropic viruses), autoimmune chronic active hepatitis, primary biliary cirrhosis, granulomatous hepatitis, and sclerosing cholangitis, inflammatory and fibrotic lung diseases (including inflammatory bowel disease, ulcerative colitis, Crohn's disease), gluten-sensitive enteropathy, Whipple's disease, autoimmune or immune-mediated skin diseases (including bullous skin diseases, erythema multiforme and contact dermatitis, psoriasis), allergic disease (including asthma, allergic rhinitis, atopic dermatitis, food hypersensitivity and urticaria), immunologic disease of the lung (including eosinophilic pneumonias, idiopathic pulmonary fibrosis, hypersensitivity, pneumonitis, transplantation associated diseases (including graft rejection and graft-versus-host-disease).

The treatment method of the present invention involves administering to a mammal the compound or pharmaceutical composition in a therapeutically effective

amount sufficient to treat the conditions or disease in question. As used herein, the term "therapeutically effective amount" means an amount that produces the effects for which it is administered. The exact dose will be ascertainable by one skilled in the art. As known in the art, adjustments based on age, body weight, sex, diet, time of administration, drug interaction and severity of condition may be necessary and will be ascertainable with routine experimentation by those skilled in the art.

Suitable routes of administration for the compound or pharmaceutical composition include, but are not limited to, oral, rectal, transdermal, vaginal, transmucosal or intestinal administration, parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, intraocular, and the like.

Other Uses for the Human JAM3 Nucleic Acids and Protein

The nucleic acids of the present invention can be used to create recombinant cell lines (either stable or transient) which express a human JAM3 protein or human JAM3 protein mutated at selected positions to determine which part of the molecule is responsible for function. The hereinbefore described recombinant cell lines can contain a tag at either the 5' or 3' end, such as, but not limited to an HA epitope, to enable monitoring of human JAM3 function/modification/cellular interactions.

The human JAM3 nucleic acid can be used to identify antisense oligonucleotide for inhibition of human JAM3 protein function in cell systems. Further, degenerate oligonucleotide may be designed to aid in the identification of additional members of this family by the polymerase chain reaction. Alternatively, low stringency hybridization of cDNA libraries may be performed with human JAM3 nucleic acids to identify closely related sequences.

The intracellular domain (amino acids 265-310) of human JAM3 protein can be used to "fish" for novel interacting partners in a yeast two-hybrid system using techniques known to one skilled in the art. Additionally, the human JAM3 nucleic acids

of the present invention can be used to inactivate an endogenous gene by homologous recombination and thereby create a human JAM3 deficient cell, tissue, or mammal. Such cells, tissue or mammals can be used to define specific *in vivo* processes which are normally dependent upon human JAM3.

Because human JAM3 protein is expressed to a low level in many tissues, it is likely that human JAM3 protein can be upregulated during pathological conditions. This expression pattern suggest that human JAM3 protein localizes to endothelial cells. However, it is certainly possible that other cell types also express human JAM3 protein. If human JAM3 protein localizes to the tight junction of endothelial cells, Applicants believe that it may play a role during metastasis. Either defective human JAM3 protein or decreased expression may not only decrease adhesion between tumor cells but also facilitate their movement through the endothelium into the vessel. Human JAM3 protein expression in the brain indicated that it plays a role in the blood brain barrier. Additionally, human JAM3 protein expression in the aorta and heart also indicates that it plays a role during conditions which display inflammatory or permeability changes such as atherogenesis and reperfusion injury.

By way of example, and not of limitation, examples of the present invention shall now be given.

EXAMPLE 1: Isolation And Cloning Of Human JAM3 Nucleic Acid

Example 1.A(1). cDNA Cloning of Human JAM3

The human JAM2 nucleic acid (Accession No. AY016009) was used to retrieve similar sequences form the expressed sequence tag (hereinafter "EST") database. GenBank™ accession numbers AW162934, H71984, BE783472, BE783711, encoding an incomplete JAM3 molecule, were assembled to form the ecto- and transmembrane domains. The final 17 base pairs of the assembled sequence at the 3' end (namely, TTACAAGAACCCAGGGA (SEQ ID NO:3)), was mapped to contig 40948-57168 within GenBank™ accession number AP001775. To complete the intracellular domain, this contig was translated in all three reading frames and the sequence penultimate to all

stop codons examined for putative PDZ motifs. From this analysis, a candidate sequence encoding the C-terminus of the human JAM3 protein was identified. A reverse oligonucleotide, was designed, 5'-TCAGATCACAAACGATGAC-3' (SEQ ID NO:4) and coupled with the forward primer, 5'-ATGGCGCTGAGGCGGCCA-3' (SEQ ID NO:5), for amplification of the full open reading frame. Human fetal brain mRNA (CLONTECH, Palo Alto, CA) was reverse transcribed with M-MLV reverse transcriptase (Life Technology Inc., Carlsbad, California) and amplified using Pfu DNA polymerase (STRATAGENE, La Jolla, CA) by cycling 30 times at: 94°C for45s, 58°C for 45s, 72°C for 90s. Highlighting of the JAM3/JAM2/JAM1 multiple sequence alignment was performed using BOXSHADE (written by K. Hoffman and M. Baron, unpublished, available through http://www.ch.embnet.org.

Example 1.A(2).Protein Expression Of Human JAM3

The ectodomain of human JAM3 protein was amplified with sense 5'-GATATCAATATGGCGCTGAGGCGGCC-3' (SEQ ID NO:6) and antisense 5'-GGTACCGTTCAGGTCATAGACTTCC-3' (SEQ ID NO:7) primers that included EcoRV and KpnI restriction sites respectively (underlined). Amplified product was obtained using the full-length cDNA as template and by cycling 20 times: 95°C for 45s, 54°C for 45s, 72°C for 45s. Following ligation into pFastBac-Fc (pFastBac available from Life Technologies and modified to express Fc as previously described in Cunningham, S.A., et al., *J. Biol. Chem.*, 275:34750-34756 (2000)) and expression in SF21 cells, secreted JAM3-Fc fusion protein was purified over protein A Sepharose. When required, the Fc component was cleaved from the recombinant JAM3 ectodomain with thrombin (as previously described in Cunningham, S.A., et al., *J. Biol. Chem.* 275:34750-34756 (2000)). For preparation of polyclonal serum, female BALB/c mice (8-week old; Harlan, Indianapolis, IN) were immunized and then boosted 3X, 28 days apart, by intraperitoneal and subcutaneous injections of 100ug purified JAM3 extracellular domain emulsified with an equal volume of Freund's adjuvant. Complete Freund's adjuvant was used for the first immunization and incomplete Freund's adjuvant for subsequent injections. Serum was collected 10 days following each boost.

Example 1.B. Chromosomal Localization, Intron/Exon Structure of JAM3

The complete human JAM3 nucleic acid (GenBank™ Accession No. AF356518; March 2001) open reading frame was used to retrieve genomic data from the high throughput genome sequence database (htgs) using the Basic Local Alignment Search Tool (BLAST, version 2.1) tool. The size of the introns and exons were compared between the human JAM1 nucleic acid (GenBank™ Accession No. AF111713) and human JAM2 nucleic acid (GenBank™ Accession No. AY016009).

Example 1.C. Northern Blot Analysis of JAM3

A 591 bp human JAM3 nucleic acid probe was amplified with Takara Ex Taq™ DNA Polymerase using forward 5'-ATGGCGCTGAGGCGGCCA-3' (SEQ ID NO:8) and reverse 5'-TAAGTGGAAAGAAGAATTGCG-3' (SEQ ID NO:9) primers by cycling 25 times at: 95°C for 30s, 58°C for 30s, 72°C for 40s. The product was radiolabeled with [α-^{32}P]dATP using the Strip-EZ DNA kit (Ambion Inc., Austin, TX) and used to probe normalized multiple tissue Northern blots (CLONTECH) under high stringency. For autoradiography, membranes were exposed to hyperfilm MP (Amersham Pharmacia Biotech., Piscataway, NJ) at -80°C.

Example 1.D. Adhesion Assay

The cell lines, HSB, HPB-ALL, RAMOS (HSB and HPB-ALL were received from Dr Bradley W. Mcintyre, University of Texas MD Anderson Cancer Center, Houston, Texas), HL-60 and K562 (HL-60 and K562 are available from the American Type Culture Collection, Manassus, Virginia) were loaded with calcien-AM (Molecular Probes Inc., Eugene, OR) and adhesion to recombinant human JAM3-Fc, JAM2-Fc and JAM1-Fc performed in 96 well plates as previously described in Cunningham, S.A., et al., *J. Biol. Chem.* 275:34750-34756 (2000). Binding to mouse IgG2a was used to subtract background. For adhesion to CHO cells, the stable expressing JAM2 cell line was used (Cunningham, S.A., et al., *J. Biol. Chem.* 275:34750-34756 (2000)). Briefly, incubation with cells was for 90 min. at 37°C in Tris-buffered saline plus 1mM each of

$CaCl_2$, $MgCl_2$, and $MnCl_2$. Following three washes at RT, adhered cells were lysed and fluorescence quantified in a Cytofluor with excitation at 485 ± 20nm and emission at 530 ± 25nM.

Example 1.E. Direct Protein-Protein Interactions

Ninety-six (96) well plates were coated with purified JAM3 ectodomain at 354nM. The recombinant ectodomain JAM-Fc fusion proteins were added at 364nM in binding buffer (described above) and allowed to interact for 1 hour at room temperature. Wells were washed with TBST (1x) followed by TBS (3x) and detection achieved with alkaline phosphatase conjugated GAM-IgG (1:2000).

Example 1.F. QrtPCR Of JAM3 In Leukocyte Cell Lines

Total mRNA from human tissues were purchased from CLONTECH or prepared from cell lines using RNeasy Minikit (Qiagen, Valencia, CA). Quantitative real time PCR assays were performed with TaqMan reagents on an Applied Biosystems 7700 Sequence Detector at the Department of Integrative Biology and Pharmacology at the University of Texas-Houston Medical School. Primers and probes were purchased from IDT, Coralville, IA. Amplicons were derived within JAM2 exon 6 (78bp), JAM2 exon 5 (71bp) and JAM3 exon 4-5 (70bp). TaqMan probes with 5'-6-FAM / 3'-TAMRA were: JAM1 (antisense, 5'-TACCCATTCCGTGCCTCACAGCTG-3' (SEQ ID NO:10); JAM2 (sense), 5'-CCGTTTGCTAGAAAATCCCAGACTTGGC-3' (SEQ ID NO:11); JAM3 (antisense), 5'-CAGCCTTCGGCACTCTACAGACAGGG-3' (SEQ ID NO:12). Amplicons were synthesized with the following combinations of primers: JAM1, sense 5'-TCCCCTGTCAGCCTCTGATA-3' (SEQ ID NO:13) and antisense 5'-GCATTTGAAGTCATGGGTGTC-3' (SEQ ID NO:14); JAM2 sense 5'-ACACATGGTTTAAGGATGGCA-3' (SEQ ID NO:15) and antisense 5'-TGAGCTGTTGGTGCTTTGG-3' (SEQ ID NO:16); JAM3, sense 5'-CTGTGCAAGTGAAGCCAGTG-3' (SEQ ID NO:17) and antisense 5'-GTTGCCATCTTGCCTACTGGTA-3' (SEQ ID NO:18). The final data were normalized to β-actin and are presented as the molecules of transcript/molecules of β-actin x 100 (%β-actin).

Example 1.G. Pull-Down Of JAM3 Interacting Proteins

Cells were surface biotinylated with EZ-Link Sulfo-NHS-Biotin (PIERCE, Rockford, IL) and lysed in Tris buffered saline (pH 7.5), 1% Triton X-100 using identical procedures to those previously described in Cunningham, S.A., et al., *J. Biol. Chem.* 275:34750-34756 (2000). For 10^6 cells, JAM2-Fc (1μg), in the presence or absence of a 10-fold molar excess of soluble JAM3 ectodomain, was used in combination with protein A Sepharose to precipitate interacting proteins. For analysis with anti-JAM3 antibody, JAM2 interacting proteins were eluted from the protein A precipitates with 50mM CHES, pH 9.0. Elutions were then immunoprecipitated with either normal mouse serum (NMS) or anti-JAM3 serum (1:500). Samples were analyzed and detected by Western blotting with streptavidin-HRP (1:14,000) and enhanced chemiluminescence (hereinafter "ECL") (Amersham Pharmacia Biotech. Piscataway, NJ).

EXAMPLE 2 RESULTS

Example 2.A. cDNA Cloning Of Human JAM3

The full open reading frame for human JAM3 protein is encoded by 930 base pairs (GenBank™ Accession number AF356518) and, at the DNA level, displays 52.2% and 52.8% identity with human JAM2 and JAM1 nucleic acids, respectively. Multiple sequence alignment of the human JAM proteins reveals 36.3% identity of JAM3 with JAM2 and 32.5% identity with JAM1 (Fig. 1). The SignalP program predicts that the JAM3 signal sequence will be cleaved after G30. The ectodomain possesses two Ig-like folds and sequence profiles lead to the conclusion that the N-terminal domain is a V-type while the membrane proximal domain is a C2-type (Smith DK, Xue H., *J. Mol. Biol.* 274:530-545 (1997); Williams AF, Barclay AN, *Annu. Rev. Immunol.* 6:381-405 (1988). The intrachain disulphide bonds that stabilize each Ig fold are predicted between C^{53}-^{115} and C^{160}-C^{230}. The C2-domain also possesses 2 additional cysteine residues, C^{160} and C^{230} that may form a second disulphide bridge within the fold. These residues are preserved in human JAM2 protein. Two N-linked glycoslyation sites are found at amino acids 104 and 192. The former is conserved only between human JAM2 protein and human JAM3 protein while the latter is preserved in all three adhesion molecules. The

short intracellular tail consists of 46 amino acids, and possesses a C-terminal binding motif for PDZ domains and a PKC phosphorylation consensus.

Example 2.B. Chromosomal Localization, Intron/Exon Structure of JAM3

The human JAM3 gene localizes to 11q25 (the long arm of chromosome 11) and the open reading frame is interrupted by 8 introns (Table 1, see below). The sequences bordering the splice-site junctions universally follow the GT/AG rule. The open reading frame spans over ≥88Kb of genomic DNA.

TABLE 1

3' splice	Coding	Exons (pb)	5' splice	Intron (bp)
nnnnnn/(N)	(1)	≥76	TCAGGG/gtgagt	≥79, 105
cttcag/GCTGCC	(2)	66	TTGAAA/gtaagt	740
tgttag/GTGTGG	(3)	144	TTCAGG/gtatga	3,470
ctgtag/GAGACT	(4)	153	TGCAAG/gtagga	398
ccacag/TGAAGC	(5)	203	ACTTTG/gtaaga	951
aaacag/GTGTTC	(6)	100	AAGTCT/gtgagt	2511
cttcag/ATGACC	(7)	130	AGAAAG/gtgagc	87
tcatag/TTACAA	(8)	55	GAGGGAG/gtaatc	321
ttgcag/GGCGAC	(9)	≥36	NNNNNN/nnnnnn	

Lower case and upper case letters denote intron and exon sequence respectively. n/N represents unknown bases. Splice site (/).

Table 2 (see below) compares the gene structure of the JAM family members. For all three genes, the intron separating the signal sequence is considerably large than the others. The remaining coding exons are fairly compact. There is remarkable conservation of gene structure in the ectodomain. Both immunoglobulin folds one and two of all three JAMs are encoded by multiple exons. The short intracellular domain of JAM3 and mRNA is made up from three exons, while JAM2 and JAM1 mRNA splice four exons together in this region.

Example 2.C. Northern Blot Analysis of JAM3

Northern blot analysis of human JAM3 nucleic acid shows expression within many tissues. More preponderant expression is seen in the placenta, brain and kidney (see Fig. 2A). A more detailed examination of the brain reveals transcription throughout all areas examined (see Fig. 2B). To determine whether human JAM3 is expressed in endothelial cells, applicants next probed mRNA derived from primary cultures of human aortic and umbilical vein endothelial cells in addition to the ECV cell line (see Fig. 2C).

The human JAM3 transcript is clearly detectable in primary endothelial cells of both origins but was not detectable in the immortalized ECV cell line.

TABLE 2

HCNC		JAM1	JAM2	JAM3
Location		1q21.2-21.3	21q21.2	11q25
EXON description				
ATG/signal sequence	**1**	**103**	**≥301**	**≥76**
Intron	*1-2*	*44,222*	*43,994*	*79,1058*
	2	**59**	**66**	**66**
Intron	*2-3*	*167*	*5,916*	*740*
1st half, Ig-fold 1	**3**	**98**	**108**	**114**
Intron	*3-4*	*252*	*3,782*	*3,470*
2nd half, Ig-fold 1	**4**	**137**	**153**	**153**
Intron	*4-5*	*282*	*4,768*	*398*
1st half, Ig-fold 2	**5**	**203**	**203**	**203**
Intron	*5-6*	*167*	*3,290*	*951*
2nd half, Ig-fold 2	**6**	**103**	**100**	**100**
Intron	*6-7*	*128*	*3,709*	*2,511*
Transmembrane	**7**	**108**	**108**	**130**
Intron	*7-8*	*231*	*3,347*	
IC,	**8**	**13**	**16**	
Intron	*8-9*	*304*	*2,890*	*87*
IC,	**9**	**49**	**43**	**55**
Intron	*9-10*	*136*	*2,257*	*321*
IC, PDZ domain/STOP	**10**	**≥937**	**≥283**	**≥36**

HGNC - Homo sapiens official gene symbol and name (HUGO Genome Nomenclature Committee); EC, extracellular domain; IC, intracellular domain; Ig-fold, immunoglobulin-like fold; ATG*, JAM1 start at bp 40-42; JAM2 start at bp 235-237; JAM3 start at bp 1-3.

Example 2.D. Adhesion Assay

Applicants previously showed that human JAM2 protein is capable of adhering to T-cell lines (see, Cunningham, S.A., et al., *J. Biol. Chem.* 275:34750-34756 (2000)). Thus, Applicants next tested the ability of immobilized human JAM3 protein to capture various leukocytes. Fig. 3 shows that JAM3 is unable to adhere to HSB, HPB-ALL, RAMOS, HL-60 or K562 cells under the same conditions that are favorable for JAM2.

Example 2.E. Direct Protein-Protein Interactions

Human JAM1 and JAM2 protein are capable of forming homotypic interactions (see, Cunningham, S.A., et al., *J. Biol. Chem.* 275:34750-34756 (2000); Bazzoni, G., et al. *J. Biol. Chem.*, 275:20970-30976 (2000)). Therefore, Applicants assumed that human JAM3 protein would maintain this characteristic. Using purified recombinant Fc-fusion protein and immobilized human JAM3 protein ectodomain, a weak binding to JAM3-Fc was detected (see Fig.. 4). However, most strikingly, when JAM2-Fc was added to the assay a very strong heterotypic interaction was apparent. Human JAM1 protein was not functional in this regard.

To demonstrate that human JAM3 protein interacts with human JAM2 protein at the cell surface, adhesion experiments were performed between immobilized JAM3-Fc ectodomain and stable cell lines of CHO expressing full length JAM2. Fig. 5 demonstrates that surface expression of human JAM2 protein confers on CHO cells the ability to bind to human JAM3 protein.

Previous studies with human JAM2 protein have demonstrated that an uncharacterized 43 kDa surface membrane protein participated in HSB cell binding (see, Cunningham, S.A., et al., *J. Biol. Chem.* 275:34750-34756 (2000)). Thus, Applicants asked whether human JAM3 protein could be a candidate for this receptor.

Example 2.F. Expression of JAM3 In Leukocyte Cell Lines

To determine whether human JAM3 protein was specifically expressed in T-cells, qrtPCR was performed in the various leukocyte cell lines used for adhesion. Expression levels were compared with those of human JAM1 protein and human JAM2 protein. Consistent with the binding assay (Fig. 3), Table 3 shows that human JAM3 protein is only found in the HSB and HPB-ALL T-cell lines, and is absent from RAMOS, HL-60 and K562. JAM2 levels are virtually non-existent throughout, whilst JAM1 expression is strong.

To demonstrate expression of human JAM3 protein at the cell surface, the inventors developed a mouse polyclonal anti-human JAM3 antibody. FACS analysis of HSB and HL-60 cells showed both specificity of the human JAM3 antibody and specific expression of JAM3 on the HSB cell surface.

TABLE 3

	JAM3	JAM2	JAM1
HSB	0.61 ± 0.11	0.00 ± 0.00	0.39 ± 0.06
HPB-ALL	0.49 ± 0.17	0.00 ± 0.00	0.41 ± 0.17
RAMOS	0.00 ± 0.00	0.00 ± 0.00	0.37 ± 0.07
HL60	0.00 ± 0.00	0.00 ± 0.00	0.28 ± 0.06
K562	0.00 ± 0.00	0.00 ± 0.00	1.46 ± 0.11

Table 3 above shows the quantification of JAM transcripts in leukocytes by qrtPCR. Total mRNA was prepared from cell lines as shown and subjected to quantitative real-time RT-PCR utilizing the 7700 Sequence Detector (Applied Biosystems) and TaqMan reagents. Numbers represent Average ± SEM from 3 independent RNA isolations.

Example 2.G. Pull-Down Of JAM2 Interacting Proteins

To confirm the strong circumstantial evidence for a direct interaction between human JAM2-Fc and human JAM3 protein expressed in HSB cells, pull-down experiments were employed. As a first step, the investigators captured the 43kDa HSB biotinylated membrane protein with JAM2-Fc/protein A. Interacting proteins were eluted from human JAM2 at pH 9.0 and subjected to immunoprecipitation. Fig. 6 demonstrates that the anti-JAM3 serum, but not the NMS specifically precipitates the 43kDa band.

Finally, Applicants tested whether soluble human JAM3 protein could act as a competitive inhibitor. In the first approach, excess JAM3 ectodomain was included in pull-down experiments. Fig. 7A shows that the intensity of the 43kDa band is significantly attenuated in the presence of soluble JAM3. In the second approach and at the level of cell adhesion, binding of JAM2-Fc to HSB cells was performed with the inclusion of soluble human JAM3 protein in the binding buffer. Fig. 7B shows that human JAM3 protein is very effective at preventing human JAM2 protein capture of HSB cells. That this inhibition was specific for the JAM2/JAM3 heterotypic interaction was confirmed by the lack of effect on VCAM adhesion, which is dependent upon integrin (Elices, M.J., et al., *Cell*, 60:577-584 (1990); Reugg, C., et al., *J. Cell Biol.*, 117:179-189 (1992)).

Example 3. Identification of Compounds and Proteins that Modulate or Interfere with JAM2-α4 Integrin Binding.

JAM2-Fc adhesion to T cell lines was performed by capture of fusion protein by goat anti-mouse coated 96 well plates as described in Cunningham et al., *J. Biol. Chem.*, 275:34750-34756 (2000) or by capture of JAM2-Fc possessing a c-terminal myc epitope with chicken anti-myc antibodies (See Cunningham et al., *J. Biol. Chem.*, Jun 17 (2002)). The HSB cells were loaded with calcien-AM (Molecular Probes Inc., Eugene OR) and re-suspended in Tris Buffered Saline (TBS) with or without the addition of 1 mM $MnCl_2$. Binding to mouse IgG2a was used to subtract background from experiments using goat anti-mouse as capture reagent. Soluble JAM3 was generated from a JAM3-Fc fusion that was digested with thrombin and purified to remove the Fc component.

Neutralizing anti-JAM3 polyclonal serum was generated in mice using human JAM3 extracellular domain as immunogen. TBC 772 is a cyclic hexapeptide (C*WLDVC*) and known antagonist of alpha 4 integrins; TBC 1194 is a control scrambled peptide (C*DLVWC*) (Vanderslice, P., *J. Immunol.*, 158:1710-1718 (1997)). For candidate drug additions, TBC 772 were added to the cells just prior to their addition to JAM2-Fc. Following adhesion for 90 minutes at 37°C, wells were washed 3 times at room temperature. Adhered cells were lyzed and fluorescence quantified in a Cytofluor with excitation at 485±30 nm and emission at 530±25.

Fig. 8A shows that TBC 772 attenuates the manganese enhanced JAM2 adhesion to HSB cells. The cation-independent JAM2 interaction with JAM3 is not affected. TBC 1194 maintains both cation-independent and Mn-enhanced components. Based upon this data, the inventors believe that JAM2 interacts with both JAM3 and α4 integrins on cells which express α4 integrins, including, but not limited to, T cells.

Fig. 8B shows an example of the dose-response effect for TBC 772. While no effect is seen on the JAM2 interaction with JAM3 at any dose, the Mn-enhanced adhesion is progressively attenuated. The IC50 approximates to 60nM.

Fig 9A shows that soluble JAM3 inhibits both the JAM2 interaction with JAM3 (TBS component), in addition to the further binding of JAM2 with α4 integrin (TBS + Mn component). The inventors believe that soluble JAM3 ectodomain would bind to the JAM2 on the well surface and prevent its interaction with JAM3 on the HSB cell surface. It is concluded that under these conditions, that JAM2 is unable to engage α4 integrin. Therefore, a specific interaction of JAM2 with cell-surface bound JAM3 is necessary before JAM2 can bind α4 integrin. Likewise, if neutralizing anti-JAM3 antibodies are used to prevent JAM2 binding to cell surface JAM3, then JAM2 is also unable to engage integrin (Fig 9B).

Based upon this data, the inventors believe that JAM2 interacts with both JAM3 and α4 integrin on cells, including but not limited to T cells. Engagement of JAM2 with

α4 is dependent upon JAM3 which suggests that JAM3 acts as a co-factor for JAM2 binding with integrin.

Example 4. Identification of Specific Integrins That Adhere to JAM2

JAM2-Fc adhesion to HSB T cells was performed exactly as described above. Neutralizing integrin antibodies against either the α4, β1 or β2 subunits were added to the reaction. Fig. 10 shows that while both the α4 and β1 antibodies are effective inhibitors of the Mn-enhanced component of the JAM2 adhesion to HSB, the neutralizing β2 antibody is without effect. As expected, none of the antibodies affect the JAM2 binding to JAM3.

Based upon this data, the inventors conclude that JAM2 specifically interacts with the α4β1 integrin, and that JAM3 interacts with JAM2 to enhance the JAM2-α4β1 interaction.

All references referred to herein are hereby incorporated by reference.

The present invention is illustrated by way of the foregoing description and examples. The foregoing description is intended as a non-limiting illustration, since many variations will become apparent to those skilled in the art in view thereof. It is intended that all such variations within the scope and spirit of the appended claims be embraced thereby.

Changes can be made to the composition, operation, and arrangement of the method of the present invention described herein without departing from the concept and scope of the invention as defined in the following claims.

WHAT IS CLAIMED IS:

1. A method for identifying a compound that binds to junctional adhesion molecule 3 (JAM3), the method comprising the steps of:

contacting JAM3 in the presence of a test compound; and

detecting binding between the JAM3 and the test compound.

2. A method for identifying a compound that modulates binding between junctional adhesion molecule 2 (JAM2) and junctional adhesion molecule 3 (JAM3), the method comprising the steps of:

contacting JAM2 and JAM3 in the presence and absence of a test compound;

detecting binding between the JAM2 and JAM3; and

identifying whether the compound modulates the binding between the JAM2 and JAM3 in view of decreased or increased binding between the JAM2 and JAM3 in the presence of the compound as compared to binding in the absence of the compound.

3. A method for identifying a compound that modulates binding between $\alpha 4\beta 1$ or $\alpha 4\beta 7$ integrin and junctional adhesion molecule 2 (JAM2), the method comprising the steps of:

contacting $\alpha 4\beta 1$or $\alpha 4\beta 7$ integrin and JAM2 in the presence and absence of a test compound;

detecting binding between the $\alpha 4\beta 1$ or $\alpha 4\beta 7$ integrin and JAM2; and

identifying whether the compound modulates the binding between the α4β1 or α4β7 integrin and JAM2 in view of decreased or increased binding between the α4β1 or α4β7 integrin and JAM2 in the presence of the compound as compared to binding in the absence of the compound.

4. A method for identifying a compound that modulates binding between α4β1 or α4β7 integrin and junctional adhesion molecule 2 (JAM2), the method comprising the steps of:

a) immobilizing α4β1 or α4β7 integrin or a fusion protein or fragment thereof on a solid support to form an immobilized binding partner;

b) labeling JAM2 or a fusion protein or fragment thereof with a detectable agent to form a non-immobilized binding partner;

c) contacting the immobilized binding partner with the labeled non-immobilized binding partner in the presence and absence of a compound capable of specifically reacting with α4β1 or α4β7 integrin or JAM2;

d) detecting binding between the immobilized binding partner and the labeled non-immobilized binding partner; and

e) identifying compounds that affect binding between the immobilized binding partner and the labeled non-immobilized binding partner.

5. The method of claim 4 wherein the immobilized binding partner is contacted with the labeled non-immobilized binding partner in the presence of JAM3 and in the presence and absence of a compound capable of specifically reacting with α4β1 or α4β7 integrin or JAM2.

6. A method for identifying a compound that modulates binding between α4β1 or α4β7 integrin and junctional adhesion molecule 2 (JAM2), the method comprising the steps of:

a) immobilizing JAM2 or a fusion protein or fragment thereof on a solid support to form an immobilized binding partner;

b) labeling α4β1 or α4β7 integrin or a fusion protein or fragment thereof with a detectable agent to form a non-immobilized binding partner;

c) contacting the immobilized binding partner with the labeled non-immobilized binding partner in the presence and absence of a compound capable of specifically reacting with α4β1 or α4β7 integrin or JAM2;

d) detecting binding between the immobilized binding partner and the labeled non-immobilized binding partner; and

e) identifying compounds that affect binding between the immobilized binding partner and the labeled non-immobilized binding partner.

7. The method of claim 6 wherein the immobilized binding partner is contacted with the labeled non-immobilized binding partner in the presence of JAM3 and in the presence and absence of a compound capable of specifically reacting with α4β1 or α4β7 integrin or JAM2.

8. A method of inhibiting JAM2-α4β1 or α4β7 integrin mediated interactions in a mammal, the method comprising the step of administering to a mammal an effective amount of soluble JAM3 or soluble JAM2 to inhibit said interaction.

9. The method of claim 8 wherein the soluble JAM3 or soluble JAM2 is in recombinant form.

10. The method of claim 8 wherein soluble JAM3 or soluble JAM2 is a fusion protein or a full length extracellullar JAM3 or JAM2 or fragment thereof.

11. A method of inhibiting JAM2-α4β1 or α4β7 integrin mediated interactions in a reaction mixture *in vitro*, the method comprising the step of adding an effective amount of soluble JAM3 or soluble JAM2 to the reaction mixture to inhibit said interaction.

12. The method of claim 11 wherein the soluble JAM3 or soluble JAM2 is in recombinant form.

13. The method of claim 11 wherein soluble JAM3 or soluble JAM2 is a fusion protein or a full length extracellullar JAM3 or JAM2 or fragment thereof.

14 A method of preventing JAM2 – JAM3 interaction in a mammal, the method comprising the step of administering to said mammal an effective amount of a compound identified pursuant to claims 1 or 2 to prevent said binding.

15. A method of preventing JAM2 mediated interaction with an alpha4 integrin in a mammal, the method comprising the step of administering to said mammal an effective amount of a compound identified pursuant to claims 3, 4 or 6 to prevent said interaction.

16. A method of preventing JAM2 mediated interaction with an α4β1 or α4β7 integrin in a reaction mixture *in vitro*, the method comprising the step of administering to said reaction mixture an effective amount of a compound identified pursuant to claims 3, 4 or 6 to prevent said interaction.

17. A method of preventing JAM2 – JAM3 interaction in a mammal, the method comprising the step of administering to said mammal an effective amount of a anti-JAM2 or anti-JAM3 neutralizing antibody.

FIG. 1

Fig 2A

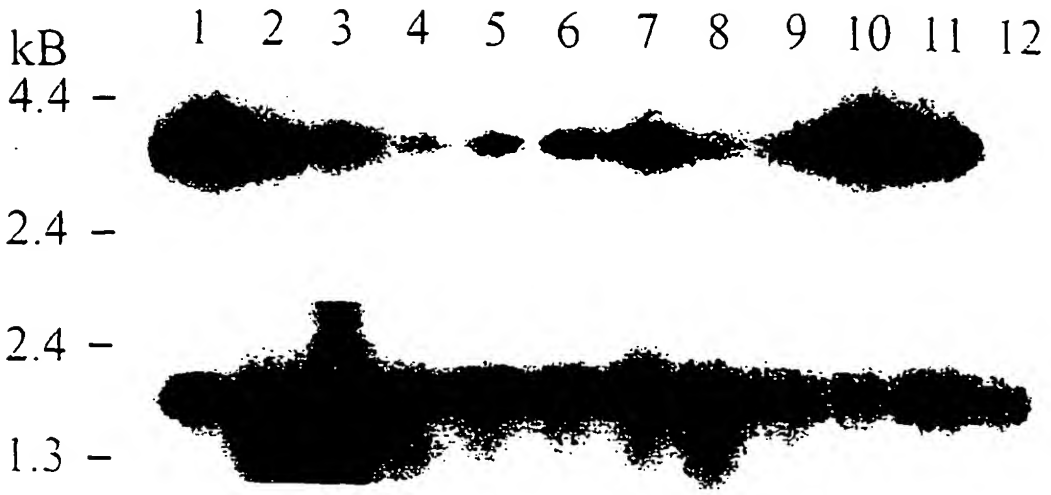
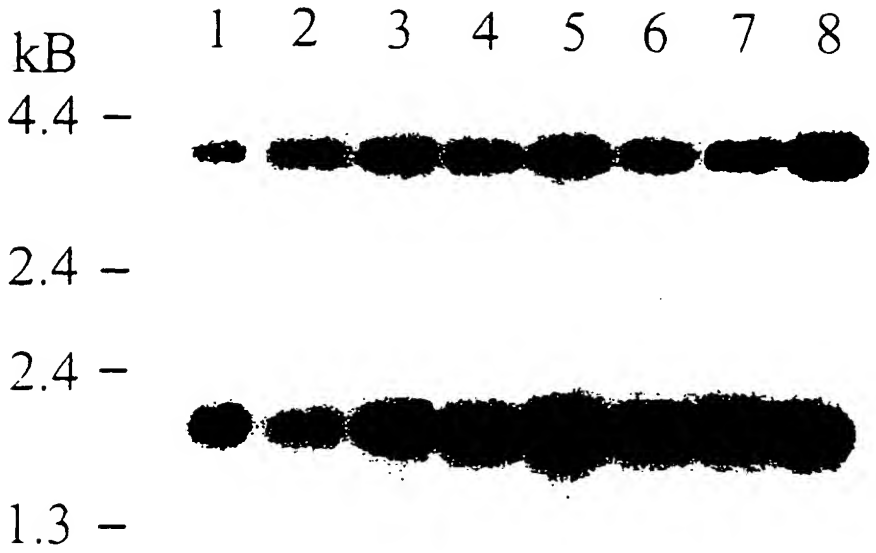


Fig. 2B



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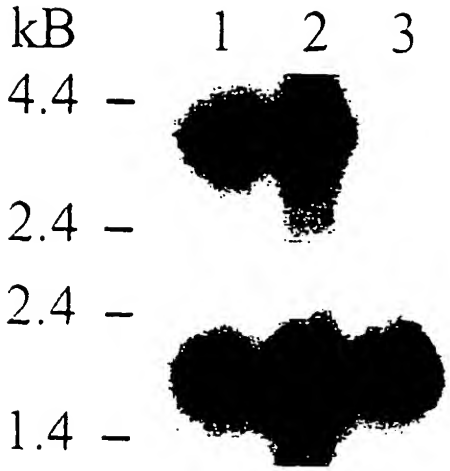

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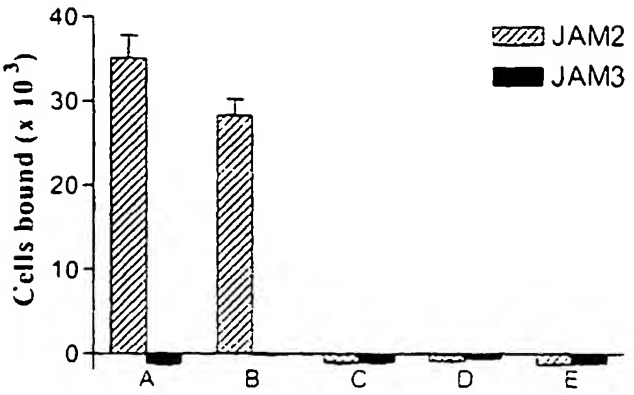

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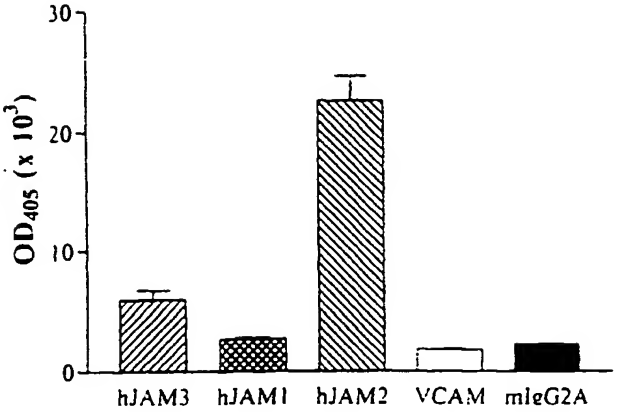

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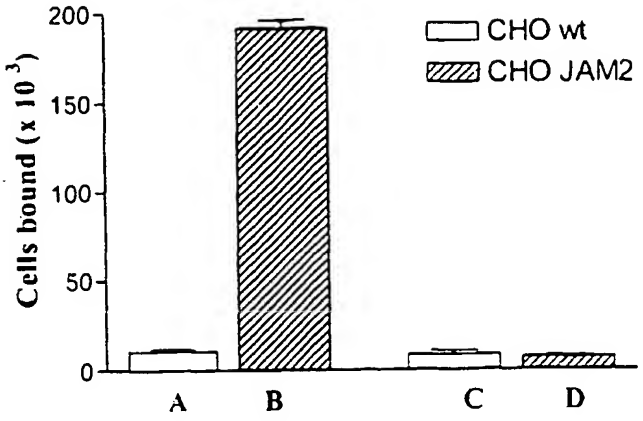

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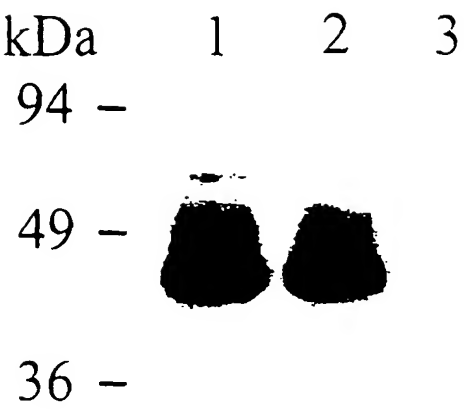

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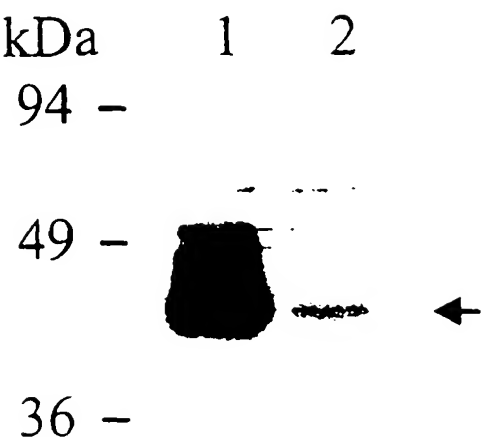


Fig 7B

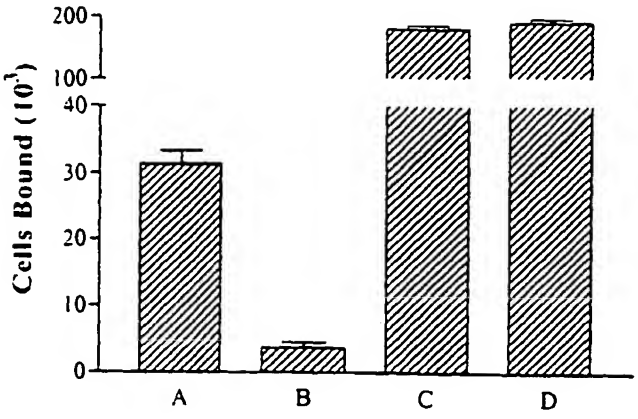
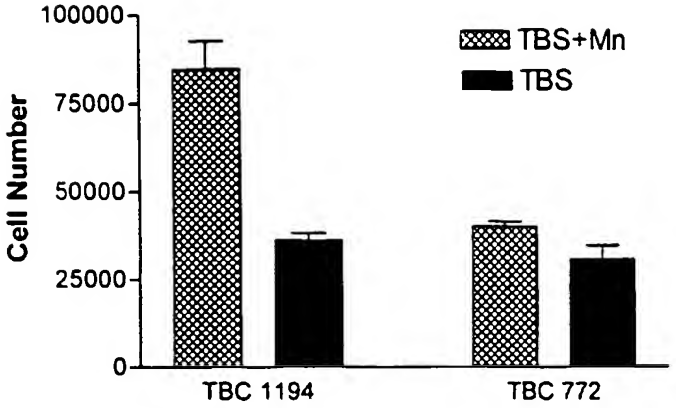


Fig. 8A



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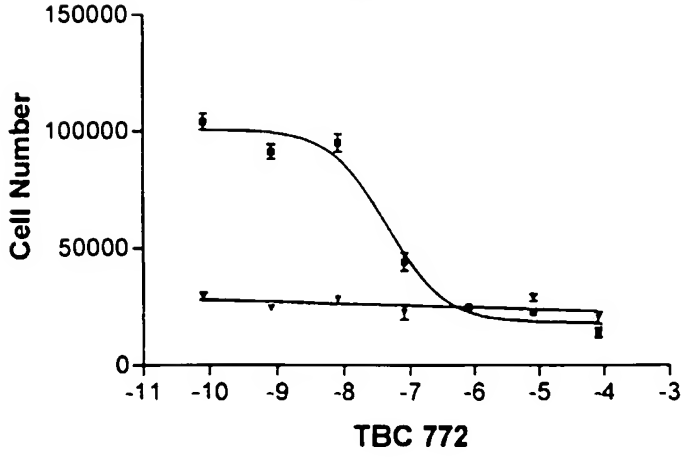
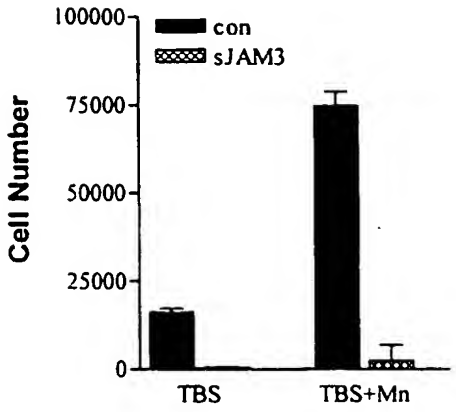


Fig. 9A



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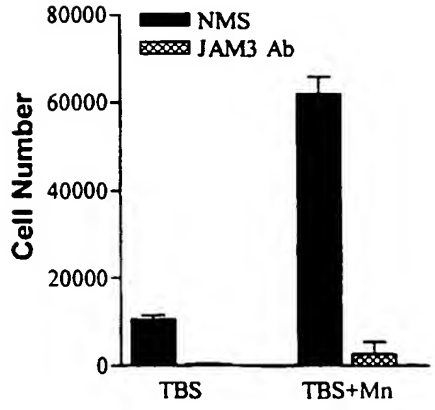
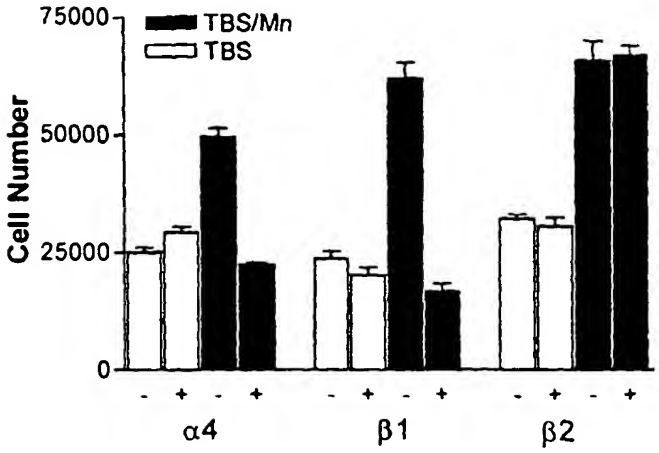


Fig.10



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